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PALEOBOTANY IN THE POST-GENOMICS ERA: INTRODUCTION[1, 2]

William L. Crepet[3] *and Maria A. Gandolfo*[4]

Today, significant biological questions are being addressed in the aftermath of the genomics revolution in ways that suggest a remarkable understanding of life is now within our grasp. Post-genomics techniques are powerful, intriguing, and full of promise. In addition to the tools grouped under bioinformatics, we are now seeing the emergence of ancillary but complementary and informative technologies that are increasingly important, including breakthroughs in optical imaging such as confocal microscopy and various microarray methodologies. Such new techniques are helping us understand the cascade of information provided by genomics and are already yielding important insights into plant biology. These include advances in understanding cell structure and function, control of gene expression, and intercellular communication that will inform our understanding of plant development and thus, ultimately, homologies. Genomics data are also vital to understanding the relationship between the organism and environment, and a better understanding of this relationship combined with better understanding of homologies will provide new insights into plant evolution, especially in the context of another post-genomics–era advance—comparative studies that allow estimations of phylogenetic relationships among extant taxa based on DNA sequence data using various algorithms. Phylogenetic context is a powerful tool for understanding many aspects of the history of life including those involving paleoecology and past distributions. Phylogenies based on gene sequence data are also increasingly being used to date key evolutionary events through various molecular clock–based models. Dating key events in the past is critical to understanding the diversity and nature of life today. Thus, in the post-genomics era, many tools of molecular biology have implications for understanding aspects of plant life that were once illuminated exclusively by fossil evidence or inferred from comparative studies of extant taxa based on morphology and anatomy.

Now, in the excitement associated with the proliferation of genomics data, there is such reliance on various molecular genetic–based approaches and so much hope assigned to future applications of such methodologies that fossil data are in some danger of either being ignored or, because many fossil identi-

[1] This and the nine articles that follow are the proceedings of the symposium "Paleobotany in the Post-genomics Era," which was held at Botany 2006 on 2 August 2006, at California State University–Chico, Chico, California, U.S.A. The symposium was supported in part by the Paleobotanical Section of the Botanical Society of America.

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fications in the literature have been inadequately justified, misapplied when they are used at the critical interface between genomics and paleontology. Thus, there is the possibility that the full value of fossil data in the context of genomics and post-genomics studies may not be fully realized at the very moment when these data might have the most significance.

This symposium issue is dedicated to emphasizing the value of the fossil record by discussing issues likely to be important in the post-genomics era. There are too many areas where fossils have importance to include here, but certain issues and subjects are covered in some depth. In the first paper, Crepet further considers the value of the fossil record in general and specifically with respect to understanding angiosperm history. Gandolfo et al. discuss the importance of fossil selection in calibrating analyses using various timing models. Nixon considers the conflict between timing suggested by the fossil record versus timing inferred from the application of molecular clock–based dating using a specific example. Such conflicts are often attributed to flaws (i.e., incompleteness) in the fossil record. Burnham considers the accuracy of the fossil record directly and addresses some widely held notions about lag time while pointing out imperfections based not on the record itself, but on the level at which it has been studied.

Integration of fossil and modern data is an important recurring element in the post-genomics era, and in this symposium this issue is approached in a variety of ways. Hermsen and Hendricks consider difficulties and benefits associated with integrating molecular sequence data from modern taxa with morphological/structural data from fossil and extant organisms in phylogenetic analyses aimed at elucidating character evolution. Endress reveals a creative and very interesting application of comparative floral organogenesis in extant taxa that has the potential to amplify the information retrievable from the fossil record, while Rothwell et al. illustrate interesting potential in the interface between the fossil record and molecular biology. They provide fossil evidence relevant to the regulation of cambial activity by auxin through time and in various clades. In so doing, they add yet another dimension to paleobotanical studies.

Biogeography and paleoecology are areas of particular and traditional importance in paleontology and ones critical to understanding the evolution of life. There is no neontological substitute for the information made available through such investigations, but modern data and analytical techniques are vital to informing these studies. Tiffney takes on biogeography in the context of post-genomics–era methodologies using phylogenetics, applications of "physiological uniformitarianism," and key fossil data in concert to evaluate certain phylogeographic hypotheses and to identify new ones. DiMichele and Gastaldo provide a comprehensive treatment of the interface between neoecological techniques and paleoecology. They illustrate how reciprocity between advances in paleobotany/paleoecological methods and modern ecological theory/methods holds great potential for a better understanding of the changes in Hutchinson's "ecological theater" through time and thus promises to impact our understanding of his "evolutionary play."

There are several themes emerging from this collection of papers. It is clear that new methodologies are critical to increasing the value of fossil evidence and, reciprocally, that fossil evidence is important in exploiting many of these advances. Furthermore, it is likely that fossil data will be increasingly important as new informative techniques emerge that we cannot now imagine, just as we could not predict the value of fossils in the context of today's techniques 30 years ago. It is also very clear that the value of the fossil record, now so informative in so many areas, is limited by the accuracy of fossil descriptions, stratigraphic settings, and identifications. In the end, it is one of the most basic aspects of paleobotany that counts the most.

THE FOSSIL RECORD OF ANGIOSPERMS: REQUIEM OR RENAISSANCE?[1]

William L. Crepet[2]

ABSTRACT

The fossil record of angiosperms has more potential than ever for contributing to the resolution of major questions in the evolution of the flowering plants due to the better understanding of the significance of leaf and pollen records and because of the increasingly complete and informative fossil record of flowers. Nonetheless, the record has fallen short of its potential (and of its potentially synergistic value) because, although it is better understood than ever, there are still problems in identifying fossils' affinities that have not been fully resolved and that have major implications with respect to determining timing in angiosperm evolution with either molecular clock–based models or minimum-age node mapping. This issue is of particular significance with respect to early angiosperm radiation, where more careful studies of existing specimens seem to have unrealized value for increasing our comprehension of floral evolution and homology, potentially in the context of strides in understanding MADS-box genes. Subjective methods with typological overtones are still often used in identifying fossils, even though phylogenetic context is available. Identification using phylogenetic context, among other things, does not obscure relative character changes within monophyletic groups, does not lend itself to facultative interpretation of affinities to suit outcomes of various models, and, thus, does not impede our understanding of angiosperm evolutionary history. Nonetheless, a reasonably good fossil record of angiosperms is emerging from the combined efforts of many laboratories and, when carefully evaluated, reveals an interesting and possibly informative pattern of flowering plant evolution. One of its most striking aspects is the rapid radiation of angiosperm taxa that are now unusually diverse around two particular times in geological history: the Turonian and Early Tertiary. Possible reasons for these intervals of rapid radiation among angiosperms will be discussed.

Key words: Cretaceous, diversification, Hymenoptera, insect pollination, MADS box, molecular clock, Tertiary, typological.

As the post-genomics era unfolds, the role of the fossil record in addressing broad questions related to plant evolution has come under scrutiny. While there are certain aspects of fossil data that are unique and cannot be replaced by comparative studies of extant taxa, the primacy of fossil evidence in what have been key areas exclusive to the domain of paleontology has been challenged. For example, the implications of fossil evidence in timing evolution, one of the principal areas of its importance, has often come into conflict with predictions derived from molecular clock–based models that sometimes produce dramatically contrasting results (Wikström et al., 2001; Crepet et al., 2004; Graur & Martin, 2004; Sanderson et al., 2004). In these latter instances, the fossil record's flaws are often invoked directly or by implication to explain the discrepancies in favor of the model's results (Burnham, 2008), or there may be a tendency to look too hard for fossils that might corroborate the predictions of such models with the danger that standards of identification might be relaxed in so doing (Crepet et al., 2004). Another manifestation of conflicts between the results of molecular-based models and the fossil record involves dramatic examples where the taxonomic affinities assigned to fossil taxa have been reinterpreted because of conflicts with predictions based on models, despite the uncertainties associated with the models themselves and without supporting analyses that might favor the suggested change in affinities (Nixon, 2008). These instances reveal trends that could diminish the impact of fossil evidence in the face of alternative methodologies before the potential value of fossil evidence is fully realized and before the accuracy of such alternative means of timing has been fully evaluated. With the possibility of controversy in such a critical area as timing, the full potential of fossil evidence in understanding evolutionary history cannot be appreciated because the potential informative value of plant fossil data depends on correlative studies that, in turn, depend on timing (i.e., models explaining evolutionary events can be evaluated for

[1] This research was supported in part by National Science Foundation grant DEB-0108369 to WLC and Kevin Nixon. The author thanks Maria Gandolfo, Karl Niklas, and Kevin Nixon for helpful comments and corrections and is indebted to Victoria C. Hollowell of the Missouri Botanical Garden for her careful editing and excellent suggestions. The author acknowledges David L. Dilcher for illustrations in Figures 1 and 8, and Michael Rothman for his reconstructions in Figures 9–11. He also thanks Wolf B. Frommer, Plant Biology, The Carnegie Institution at Stanford for valuable discussion.

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consistency with the fossil record) or on paleobiogeographic/paleoecological context that, again, requires an accurate time frame. This paper is one in a collection of symposium contributions aimed at examining the fossil record of plants to illustrate how it might continue to be of value in the face of dramatic new developments in molecular genetics and their applications (e.g., Downie & Palmer, 1992; Parkinson et al., 1999; Chaw et al., 2000), and at providing examples of new applications of fossil data in this context. Due to obvious space limitations, it is by no means a comprehensive treatment of all aspects of the integration of fossil and modern data, but is intended to underscore the importance of fossils and their unrealized potential at a time when excitement generated by alternative, powerful techniques may obscure the value of fossil data and ultimately diminish its contributions to addressing questions of interest and importance. This would eventually have the ironic result of limiting the impact of important breakthroughs in molecular genetics by removing a valuable set of tools from interpreting and using such approaches.

Certainly, the fossil record does have uncontested and exclusive domain in its potential for revealing taxonomic history in an ecological or a paleobiogeographic context. Thus, the fossil record empowers an entire class of studies of evolution/development where, in addition to timing and relationships, ecological context is also important for understanding evolution (e.g., Jablonski, 2005). While studies of molecular genetics have the potential to illuminate key events in evolution by identifying genes and clarifying genetic control mechanisms associated with timing in development (in addition to the taxonomic distribution of these genes), critical aspects of the relationship between evolution and development would be invisible to studies that focused exclusively on contemporary species (e.g., correlations between environment or key environmental components [s.l.] and character innovations [e.g., Crepet, 2000; Grimaldi & Engel, 2005; Jablonski, 2005; Labandeira, 2006]). Thus, separate from its historical importance in attaining our present understanding of the history and evolution of life, the fossil record has unique properties that are important to a deeper understanding of evolution depending on the nature of the particular fossil record and how it has been interpreted and applied.

The Fossil Record

Value of Fossil Evidence

The fossil record provides information relevant to understanding plant evolution and diversity in a number of important areas including the following: (1) Determining the pattern of taxa appearances through time—this allows tests for consistency with various evolutionary ecological hypotheses via comparisons with hypothesized related parallel events. (2) Determining minimum times of appearance of characters or taxa—especially important in the context of depositional environment. (3) Identifying morphoclines—transitional states that form a sequence linking two disparate homologous conditions where transitional types may be difficult to hypothesize (verifiable in phylogenetic context). (4) Identifying mosaics or links among taxa, such as extinct taxa that include combinations of characteristics now found in different taxonomic groups and, therefore, are possibly useful in linking groups (i.e., missing links through the combination) or in checking existing phylogenies for consistency (i.e., if the combination of characters verified in a fossil taxon cannot occur on a phylogenetic tree, then that tree is incorrect). These taxa, as informative as they might be, are invisible in cases where typologists, baffled by their unique nature, attempt to identify them without including them in phylogenetic context. This approach has obscured the observation of evolution within lineages and opens the door for controversy about affinities by making determinations necessarily, to at least some degree, subjective. (5) Observing distribution changes through time, allowing for a check on paleobiogeography patterns and their implications for explaining modern distributions (Raven & Axelrod, 1974). (6) Reconstructing paleoecology/climatology. (7) Examining the evolutionary implications of depositional environments. (8) Identifying extinct diversity. (9) Observing adaptive radiations and mass extinctions (e.g., turnover rates).

As implied above, the plant fossil record, which includes only morphology and structure with very occasionally preserved chemistry, might be considered one of two independent sets of data relevant to plant history, evolution, and relationships. The other is based on extant taxa and includes comparative morphology, structure, and molecular genetics data. With respect to analyzing the significance of these separate sets of data, complementarities now exist in various combinations of fossil and modern data through phylogenetic analyses, in assessments of congruence checks between fossil mosaics and phylogenies generated without fossils, by comparisons between apparent character transformations based on fossil intermediates and those implied by phylogenetic context or even by underlying molecular genetics, and by general comparisons between patterns based on comparative analysis of extant taxa with their implied sequences of evolution and patterns observed in fossil

history. Analyses that include some synthesis between these data sets aimed at understanding evolution have changed progressively with the development of new methodologies in phylogenetic analysis (e.g., Nixon, 1999, 2003) and advances in molecular genetics. There are, however, enduring first principles upon which effective interpretations of fossil data depend. The most fundamental of these is accuracy in identification of the fossils (Gandolfo et al., 2008). Without such accuracy, none of the potential of fossil taxa can be realized. Timing, a critical example, follows from the pattern of appearance of taxa that are accurately identified. This pattern in time has enormous value beyond simply determining minimum times of appearance of taxa or characters or as references for molecular clock–based models (Crepet, 1996; Magallón et al., 1999; Crepet et al., 2004; Friis et al., 2006).

IDENTIFICATION OF FOSSIL TAXA

With respect to maximizing the potential informative value of the fossil record in any of the areas mentioned above (or in any of its usual applications), it is the seemingly simple and straightforward process of taxon identification that has caused a great deal of controversy in interpreting the fossil record of plants, particularly, but not exclusively, angiosperms, and has also prevented the full realization of the potential informative value of the plant fossil record. For vascular plants, the fossil record is further complicated by the fact that entire organisms in reproductive state are seldom preserved with component organs in organic connection. Often, only some parts of particular taxa are known as fossils. This phenomenon (i.e., that of missing morphological/structural characters) has significant implications for the identification of fossil taxa that are the foundation for other inferences and insights that may be derived from fossil evidence in the areas noted above (Nixon & Davis, 1991; Nixon, 1996). The potential associated with a pattern of evolution of taxa set in chronology and ecological context cannot be realized unless fossil identifications are precisely accurate or precisely as accurate as possible because affinities based on subsets of characters are, after all, only best estimates.

Reliable identification of fossils is more important than ever in light of widely accepted phylogenies based on molecular data that imply a sequence in timing (relative appearances) due to their hierarchical nature (i.e., creating a possible subliminal tendency to look for what might be expected in the fossil record as mentioned above). Facultative interpretations of fossils' affinities, easier to do with less rigorous methodologies for fossil taxon identification and inspired, whether subliminally or not, by phylogenies based on molecular sequence data, would be harmful to the scientific method because such interpretations might effectively eliminate the independence of fossil evidence as a corroborative instrument by linking expectations to outcomes in the realm of identities. Thus, the significance of the fossil record would be diminished, and the test for congruence between two independent sources of data, which should ideally strengthen our understanding of evolutionary history, would be compromised. Proof against this eventuality would be a careful, open methodology for taxonomic affinity determination—a methodology that, ideally, might take into consideration and compensate for challenges in describing and classifying fossil taxa whose character associations reflect evolutionary change (that is, those different from their closest modern relatives). In other words, a methodology that would allow for precise placement of extinct taxa.

PHYLOGENETICS AND IDENTIFICATION

Cladistics or phylogenetics has made it possible to identify fossils through placement in phylogenetic context (e.g., Donoghue et al., 1989; Nixon, 1996). Thus, affinities of extinct taxa may be identified precisely and with objectivity, even though they would be difficult to determine through comparisons of tables of characters in cases where the fossil taxon's characters do not exactly correspond to the characters found in any possible modern relatives. In addition, the evolutionary significance of such fossils can be evaluated as changes in character-complements within monophyletic groups, which can be tracked because relationships among these fossil taxa are revealed through phylogenetic context. Phylogenetic context also focuses investigators on taxon-defining synapomorphies.

IDENTIFICATION METHODOLOGIES: HISTORICAL PERSPECTIVE

Previous to and leading up to the advent of phylogenetic methodology, there has been an evolution of identifying methodologies throughout the 20th century that is perhaps most easily illustrated in angiosperm paleobotany. One can posit that the evolution of identifying methodologies began with the criterion of general similarity in what were considered taxon-defining characters and, in the absence of phylogenetic methodology, proceeded to what has amounted to an essentially typological method of identification involving gross character comparisons via tables. The latter approach, initiated in response to the need for a more objective identification methodology, has been used historically

in conjunction with increasingly rigorous standards for describing fossils. While not ideal, it is still sometimes employed despite the fact that the weaknesses of such an approach are now obvious (by depending on congruence of character associations for taxonomic placement, the approach cannot deal effectively with extinct taxa characterized by unique combinations of characters without subjective interpretation). In essence, this method could result in shoehorning fossil taxa into particular extant groupings defined by strict character associations (typology). This methodology obscures the evolutionary process by denying or minimally, by obscuring, one of its fundamental aspects—character association changes. This tabular character-matching method then, by necessity, also involves subjective assessments of affinities in cases (most depending on the taxonomic level and age of the fossil taxon in question) where the match is imperfect. Thus, these methods have limited the impact of fossil evidence in plants, particularly in angiosperms, given that entire suites of characters are usually not available for analysis.

In contrast, phylogenetic context provides an alternative methodology for identifying extinct taxa that do not precisely conform to the character complexes now found in their closest modern relatives (e.g., Herendeen & Crane, 1992; Crepet & Nixon, 1998b). In addition to representing an optimal means of placing fossil taxa, precision identification through phylogenetic analysis is also the best way to cope with inevitable differences of opinion that characterize the scientific community by reducing the level of subjectivity inherent in other methodologies. These are inevitable in the face of the limited numbers of characters available in fossils and, frustratingly, often involve fossils of unusual potential significance. Nonetheless, such disputes, while relatively common and potentially distracting, are important correlates of the scientific method and, if conducted within the boundaries of phylogenetic methodology, are resolvable within the limits of the relevant fossil evidence. Indeed, phylogenetic context is the only reasonable method for resolving such differences because alternatives represent a regress to methods abandoned years ago by systematists because of the well-known flaws in fundamentally subjective approaches (e.g., angiosperm systematics in 1960), and, to reiterate, determination of affinities by comparisons of characters through tables is inherently subjective because there is no logical way to deal with incongruities that are the norm and not the exception when dealing with fossils (at any but the higher, and often less informative or relevant, taxonomic levels).

Two Case Studies: Cretaceous Monocot Flowers and an Early Cretaceous Angiosperm

There are many examples of differences of opinion on the affinities of important fossils. Two, in particular, are appropriate in the context of this paper: (1) reports of certain Cretaceous monocot flowers and (2) reports of a unique Early Cretaceous angiosperm. These illustrate the differences between what might be characterized as subjectively based disagreements on affinities and ones founded on conflicting results of alternative phylogenetic analyses.

The monocot example is a case of a challenge to an assignment of affinities involving a number of Cretaceous taxa, exemplified by *Mabelia* Gandolfo, Nixon & Crepet (Gandolfo et al., 2002; Figs. 2A, B, 10A), that have been placed with modern Triuridaceae on the basis of phylogenetic analysis. Although the triuridaceous affinities have been effectively universally accepted by monocot specialists (e.g., Chase, 2004) and experts in modern Triuridaceae (e.g., Rudall, 2003) on the basis of phylogenetic placement of these fossils, Friis et al. (2006) question such affinities. Their challenge is based on differences in pollen morphology between the one modern taxon of Triuridaceae for which pollen morphology is known and the fossil taxa, and on the presence of a staminal column in one of the fossil taxa. They consider the staminal column found in some of these fossil taxa as a more generally ANITA clade dicot character (Friis et al., 2006) and not one found in modern Triuridaceae. In fact, the character assemblage of the fossil triuridaceous taxon *Mabelia* reflects remarkable congruence with those of taxa found in the modern family Triuridaceae (Gandolfo et al., 2002). However, its pollen is monosulcate and, thus, more generalized than the monosulcate-derived pollen found in the only modern taxon of Triuridaceae (out of 34) for which pollen has been described (Furness et al., 2002). Furthermore, staminal columns are actually well known in the modern family Triuridaceae (Maas & Rubsamen, 1986: fig. 14h; Maas, 1988: figs. 3, 10a). Suggestions of alternative affinities with more generalized ANITA dicots have not been supported by an evaluation/criticism of the original matrix of Gandolfo et al. (2002) on which triuridacean affinities have been assigned. Thus, this difference in interpretation by Friis et al. (2006) does not include, nor is it based on, a contravening phylogenetic analysis that would support the assertion that the fossil has more generalized non-triurid affinities (Friis et al., 2006). In the absence of an alternative analysis based on accurate characterization of modern and fossil characters, assertions of alternative affinities become subjective and an extension of the character-matching

or phenetic approach used widely before phylogenetics was available. The difference in interpretation seems based on only the alleged incongruence in characters between the fossil and modern taxa. These alleged instances of incongruence are misplaced given that at least one of the characters actually does occur in modern triurids and that the pollen differences, considering their nature and the severely limited extent to which pollen morphology is known in modern members of Triuridaceae (i.e., similar pollen could ultimately be found to exist among the 33 taxa of living triurids for which pollen morphology is now unknown), are in any case consistent with generally accepted trends in pollen evolution as well as with the well-documented tendency for pollen morphology to temporally lag floral morphology as documented in numerous Cretaceous taxa (see Friis et al., 1994; Zhou et al., 2001; Hermsen et al., 2003; Crepet et al., 2004). Just as original identifications of fossils via this method of character matching have been historically difficult because they do not accommodate evolutionary change within (monophyletic) lineages, criticisms of this type are similarly based on methodologies that do not allow for the possibility of differing rates of evolution among characters found in particular clades. So, in this case, according to the criteria suggested by Friis et al. (2006), pollen would have had to evolve at the same rate as the other floral characters in order for the fossils to be defined as triurids (also assuming that no monosulcate pollen types will be found in any of the 33 species of extant triurids for which pollen is now unknown). In this regard, it is striking that some orchids might not be defined as orchids under such a methodology because they have monads or tetrads instead of polyads (Pacini & Hesse, 2002).

A good example of a disagreement on affinities taking place within the framework of phylogenetic analysis is the Lower Cretaceous fossil taxon *Archaefructus* Sun, Dilcher, Zheng & Zhou, a fossil with implications as to early angiosperm floral morphology and perhaps to the origin of flowers (Figs. 1A, B, 8A). It is an unusual angiosperm, having naked, unisexual reproductive structures that are apparently not organized in flowers, with affinities either to all of the angiosperms (Sun et al., 1998, 2002) or, with a different interpretation of the reproductive organs as secondarily simple due to reduction, to the Nymphaeales (Friis et al., 2003). This difference of opinion has taken place entirely within the context of phylogenetic analysis (compare Sun et al., 2002 and Friis et al., 2003). The challenge to the original placement of *Archaefructus* at the base of all other angiosperms was based on a reanalysis of the Sun et al. (2002) matrix following the addition of another existing nymphaeoid taxon *Cabomba* Aubl. (Friis et al., 2003). The methodology was explicit and can be objectively evaluated. It happens that there was an error in coding leaf characters in Cabombaceae in the Friis et al. (2003) paper (Cabombaceae were coded as having dissected leaves when, actually, they have both dissected submerged leaves and entire-margined floating leaves; Nixon, 2006). When corrected and the modified matrix reanalyzed, there is no support for the inclusion of *Archaefructus* within Nymphaeales (Nixon, 2006). While this particular challenge to the affinities of a fossil taxon may have been based, at least partially, on a coding error, the point is that the explicit nature of phylogenetic context allowed for an evaluation of the original identification by peers and also permitted an evaluation of the criticism. This is a good example of a healthy difference of opinion that is supported by critical evaluation, just as the original identification was supported by such evaluation. Nonetheless, the issue of affinities of *Archaefructus* is not fully resolved. Limited numbers of characters are known for the fossil taxon, keeping its actual affinities controversial. Recent assertions that some of the reproductive branches of similar, presumably closely related taxa bear both carpels and stamens (an interpretation that would make the reproductive branches inflorescences) complicate the issue (Ji et al., 2004). However, this interpretation is not convincingly supported by the illustrations purporting to reveal such bisexual fertile axes, which seem instead to reflect several layers of plant organs compressed together. This is an important issue that can only be resolved through further empirical study of the fossils in question followed by critical analysis. It focuses on the importance of first principles in paleobotanical studies (i.e., that the fossil taxon's characters should be explicitly understood and clearly demonstrable based on the study of the fossil).

Broader Relevance to Angiosperms

The issues above play out in important ways that are current with respect to a set of evolutionary mysteries that hold a commanding position among those concerning plant history. The circumstances surrounding the radiation of angiosperms comprise some of the most addressed and notably unresolved questions in plant evolution. There have been numerous articles on the many facets of angiosperm evolution, and many of these have contributed to our understanding of the questions associated with angiosperm history. These questions include: (1) ancestry—still famously unresolved with underlying homologies (to be revealed by molecular genetics studies or by yet-to-be discovered fossil evidence) being key to its resolution, and its correlate, the origin

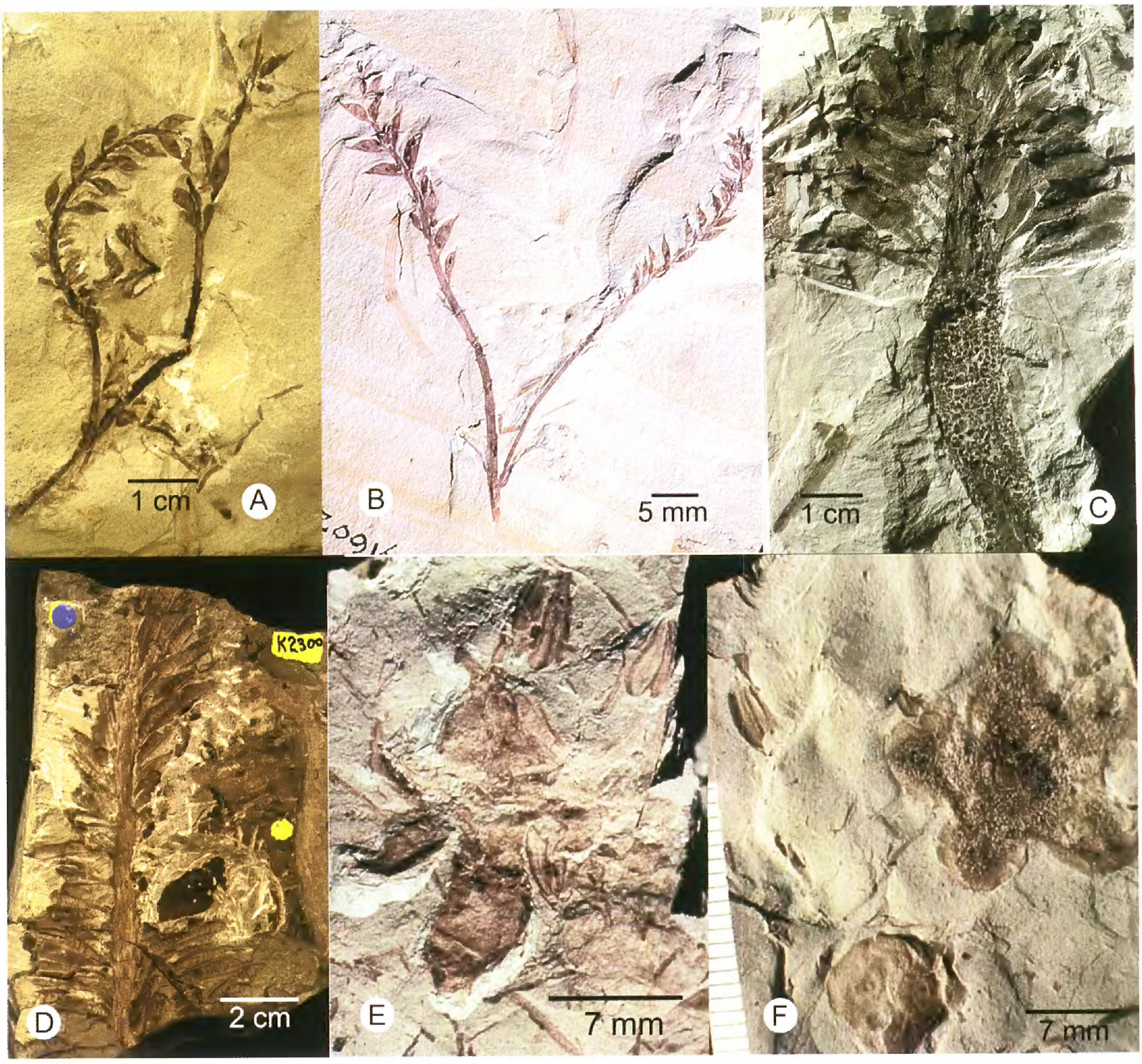

Figure 1. A, B. *Archaefructus* fertile branches (Nanjing Institute of Geology and Paleontology collection numbers SZ0917 and SZ0916). —A. Note that stamens subtend carpels on the branch on the left, and that while the fertile branches are subtended by bracts, there are no bracts or obvious floral envelope parts subtending the carpels or stamens along the fertile axes. —C, D. *Archaeanthus* Dilcher & Crane (University of Florida collection numbers UF15706-3084 and UF15703-2300): fruits from the Cenomanian of Kansas, U.S.A. Mature carpels are helically arranged on elongate axes. E, F. Compressed dicot flowers from Rose Creek, Nebraska. —E. Note stamens and carpels with separate styles and stigmas (UF15703-4026). —F. Note the radial symmetry and the apparent nectary ring and partitions separating the carpels (UF15713-3429). A–F with permission of D. L. Dilcher, Florida Natural History Museum.

of angiosperm-defining characters in ecological context; (2) within angiosperm relationships—long problematic due to convergence and homology problems and now the domain of molecular systematics (a possible escape route from convergence issues but perhaps one not yet fully resolved); (3) the dramatic rise of angiosperms to numerical species dominance and possible reasons for it; and (4) the history of angiosperm distribution patterns. There is a great deal of enthusiasm about the prospects for homology resolution in the area of molecular genetics (e.g., Theissen et al., 2000, etc.), especially given that morphological intermediates may never have actually existed, which might account for the difficulty in identifying angiosperm outgroups.

These questions have been addressed using new applications of fossil evidence. New analytical methods continue to provide hope that they will be successfully resolved. In posing the question as to how the potential of fossil evidence may be realized with respect to angiosperm history, it should again be kept in mind that reliable and appropriate fossil evidence is required, i.e., the identifications and ages of fossils must be accurately determined, and, likewise, the nature of the fossil evidence must be appropriate to the questions being addressed (e.g., questions regard-

ing flowers for evolution of pollination). In addition, methodologies must appropriately compensate for weaknesses in the fossil record (an area itself that is subject to question) in, for example, timing (minimum age node mapping or molecular clock–based models) or in phylogenetic placement of the fossils.

With respect to the questions above, it is interesting to review changing approaches to the study of angiosperm fossils because the fossil record has fallen short in some areas. One of the issues affecting our understanding of angiosperm evolution in historical context has been the unreliability of the fossil record. Studies of angiosperm fossils have been plagued by poor identification methodologies and limited by the incomplete nature of the fossil record. Nonetheless, there has been a historical trend toward increasingly rigorous methods for angiosperm fossil identifications resulting in what has now become a much more informative fossil record. With respect to angiosperm fossil identification, an important revolution of sorts was already underway in the late 1960s. New criteria were being applied in the identification of angiosperm leaves by Hickey (1971), Dilcher (1974), and Hickey and Wolfe (1975), while Doyle (1969), building on the careful stratigraphic palynology of Brenner (1963) and employing careful pollen character analysis developed with Walker (Walker & Doyle, 1975), LeThomas (1981), and others, began a comprehensive look at angiosperm pollen radiation (see also Muller, 1981). Thus, the groundwork was being laid for a better understanding of angiosperm history, an area of considerable confusion prior to that time because of rampant misinterpretations of angiosperm fossils that often erred in the direction of assigning modern affinities to fossils where such assignments were unwarranted. And, as noted above, methodologies evolved to include careful comparisons of character complexes that were more reliable than previous methods but that have aforementioned limitations in the face of better approaches afforded by phylogenetic context. With respect to the incomplete nature of the fossil record, phylogenetics and minimum age node mapping provide a means of filling in gaps as do various molecular clock–based models for determining timing.

The Value of Flowers

Given that the background of extensive leaf and pollen records and their increasing value as criteria for fossil identifications have become more rigorous, the advent and continuing investigations of fossil flowers have added significant critical mass to the angiosperm fossil record. Furthermore, flowers add several important dimensions to the record. They embody many taxon-defining characters and reveal aspects of past angiosperm reproductive biology, an aspect of angiosperms that has often been invoked to explain their relative success (Grant, 1949; Raven, 1977; Regal, 1977; Burger, 1981; Crepet, 1984; Crepet & Friis, 1987; Friis & Crepet, 1987; Eriksson & Bremer, 1992). A great deal of hope has been associated with the prospect of the recovery of a reasonably accurate fossil record of flowers as a remarkable flower record has been unfolding. While historically sporadic studies of fossil flowers have been a characteristic of angiosperm paleobotany (e.g., Conwentz, 1886), sustained efforts to find and describe fossil flowers began with investigations of compressed Tertiary flowers (e.g., Crepet et al., 1974, 1975; Crepet & Dilcher, 1977; Crepet & Daghlian, 1980; Crepet & Taylor, 1985, 1986; Crepet & Nixon, 1989a, b), followed in short order by investigations of lower Middle and Late Cretaceous fossils, represented by a variety of preservational modes (Dilcher et al., 1976; Tiffney, 1977; Basinger & Dilcher, 1984; Crane & Dilcher, 1984; Dilcher & Crane, 1984; Crepet & Friis, 1987; Friis & Crepet, 1987; Crepet & Herendeen, 1992; Crepet et al., 2004; Friis et al., 2006). As a result, a pattern of evolution of flowers has now emerged that can be reviewed with the fossil records of other plant organs as well as fossil insects that are potential pollination vectors. This collective "biotic" approach can provide insights into the evolution of reproductive biology in the angiosperms. In addition, this record of flowers, in the contexts of the carefully vetted leaf, fruit, and pollen records, provides a comprehensive record of angiosperms that can be compared to the hypothetical sequences of taxon appearances implied by phylogenies based on extant taxa and to hypotheses of timing in angiosperm history derived from various molecular clock–based models (e.g., Crepet et al., 2004). In addition, the flower record provides insights into the evolution of pollination mechanisms in angiosperm history and further illuminates relative rates of evolution among different plant organs, thus increasing the value of the fossil records of dispersed non-floral organs. This kind of information also provides insights into angiosperm reproductive strategies during key periods during their diversification.

Timing Angiosperm History

An important aspect of interpreting the significance of the history of the angiosperm fossil record and one that was once an uncritically accepted correlate of that record is the timing implied by it. Insights into questions that may be addressed by fossil evidence depend on correlation consistency with various ecological/evolutionary hypotheses, distributional

changes (Tiffney, 2008), and character evolution and its significance, and these depend on accurate timing. While timing is implied by the fossil record, the fossil record is far from perfect, and assessing the accuracy of timing implied by the fossil record in the context of molecular clock–based models has become an interesting, important, yet controversial issue (see Crepet et al., 2004; Sanderson et al., 2004; Nixon, 2008). There are a number of approaches to determining timing with fossil data. First are direct inferences from raw fossil data (i.e., data accepted from literature), second are inferences from the carefully screened (for reliability) fossil record, and finally, inferences based on various models, notably minimum age node mapping or molecular clock–based models. Of course, each of these approaches has strengths and weaknesses (e.g., uncritically evaluated literature can be misleading, critically evaluated literature may leave gaps in taxon histories, and models fill in gaps, but methodologies are in transition and their reliability controversial), but whatever the methodology employed, a more accurate and complete fossil record would and will enhance our understanding of timing. Thus, even with respect to establishing accurate timing, and in the face of a radically improved fossil record of angiosperms, the major questions surrounding angiosperm history are only partially resolved.

The Fossil Record of Angiosperms

In light of the discussion above, a review of the carefully vetted fossil record of angiosperms (i.e., fossils found to have synapomorphic characters defining the taxa to which they have been assigned or to have had their affinities determined directly in the context of phylogenetic analyses) that integrates new discoveries does reveal some interesting aspects of their history that have relevance to major unresolved issues in angiosperm evolution. There has been a recent analysis of the fossil angiosperms based on the criteria of synapomorphy or phylogenetic context (Crepet et al., 2004) and also a more recent review of the fossil record of angiosperms (Friis et al., 2006) that takes these criteria into account to some degree in its summary of angiosperm history. Friis et al. (2006) also raise a number of issues worthy of discussion, and these will be or have been considered throughout this paper (e.g., monocot fossil record, etc.). The following discussion of the angiosperm record (taxon appearances and character appearances) is based principally on flowers but is congruent with other fossil organs as noted in the relevant discussions. This discussion incorporates results of earlier reviews of the early fossil record of angiosperms. There is emphasis on Turonian (89–93.5 million years ago [Ma]) and younger angiosperm fossils in the context of the earlier fossil record of angiosperms as summarized and discussed in previous papers (Friis et al., 1986, 1997; Crepet et al., 2004; Friis et al., 2006). Key Early Cretaceous taxa, however, are discussed at length in the contexts of the overall fossil record of flowering plants and of the Turonian reports (when these are deemed to be more reliable evidence than the earlier reports of the same taxa) and how they have been investigated.

Importance of the Fossil Flower Record of the Turonian

As with new floral evidence from all Cretaceous and Tertiary intervals, Turonian data have supplied a wealth of information of angiosperm history and, given generally accepted patterns of angiosperm diversification, represent the angiosperms at an important moment in their history. These fossils are remarkable for their quality of preservation (e.g., Staedler et al., 2007) and document surprising diversity in angiosperms at a relatively early time (depending on the taxon). In addition, they reveal that certain characters and taxa had yet to evolve. These Turonian fossils include numerous initial appearances, changing our view of timing in angiosperm history in significant ways, especially with regard to the rosid-asterid radiation and also, more broadly, with respect to adaptations for insect pollination.

Timing: Earliest Evidence of Taxa and Characters Represented by Turonian Fossils

The following taxa appear first in Turonian deposits (consistent with the Angiosperm Phylogeny Group [APG] II system except as noted): Nymphaeaceae s. str., Monocot megafossils, putative Magnoliales, Caryophyllales, Hamamelidaceae, Altingiaceae, Hydrangeaceae, Iteaceae, Fagales, Rosaceae, Brassicales, Malphigiales (Clusiaceae), Myrtales, Melastomataceae, and Ericales (Ericanae) (Crepet et al., 2004).

The Fossil Evidence

Nymphaeaceae

The Nymphaeaceae comprise an interesting family placed at the base of angiosperms by molecular systematics analyses and reported based on leaves and other flowers from other sediments including sediments earlier than Turonian deposits (Friis et al., 2001, 2006). The fossil record of Nymphaeaceae is unsettled in the sense that there is some level of

controversy around each of the reports of nymphaeaceous flowers and inflorescences. The possibility that an unusual nymphaeaceous taxon is represented by *Archaefructus* (Fig. 1A, B) has been discussed above, and it is too controversial to include this taxon in the fossil record of Nymphaeaceae. More plausible reports of Nymphaeaceae are based on mesofossil evidence that includes a putative flower of Nymphaeaceae from 100 Ma sediments of the Early Cretaceous (Friis et al., 2001). However, its assignment to Nymphaeaceae s. str. does not withstand rigorous phylogenetic analysis using the same matrix employed by Friis et al. (2001; adapted from Les et al., 1999) and including only those characters available in the fossil as opposed to those inferred characters figured by Friis et al. (2001; Gandolfo et al., 2004). Upon phylogenetic analysis using the Les et al. (1999) matrix originally employed by Friis et al. (2001), results indicate that affinities are equally compatible with Illiciaceae as well as with other angiosperm families, suggesting that *Microvictoria* Gandolfo, Nixon & Crepet (Figs. 2C, D, 10B), described by Gandolfo et al. (2004), is the only unequivocally strictly nymphaeaceous fossil from the Early Cretaceous. Nonetheless, the putative nymphaeaceous fossil taxon described by Friis et al. (2001) has significant implications that are relevant to the age of Nymphaeaceae because of its equal compatibility with Illiciaceae, which suggests the node that includes both Nymphaeales and Illiciales can be dated at 113 Ma which is consistent with phylogenetic analyses (Crepet et al., 2004; Friis et al., 2006). Thus, the fossil nymphaeaceous taxon *Microvictoria*, while the earliest confirmed record, probably does not reflect the actual age of the family, which is projected at least 113 Ma by minimum age node mapping (Crepet et al., 2004).

Another perspective on the age of Nymphaeaceae that challenges both reports is derived from application of molecular clock–based models (Yoo et al., 2005). The challenge is based not on the morphology of fossils, but on the fact that they dramatically alter the outcomes of model-based angiosperm dating if they are included as calibration points (Yoo et al., 2005). The conflict with model-based analyses brings up issues involving the accuracy of the applications of at least some of these models and is discussed in detail in Nixon (2008).

MONOCOTYLEDONS

Flowers of Turonian-age Triuridaceae have already been discussed above. They exist in the context of an interesting, relatively sporadic, and somewhat controversial fossil record. Based on minimum age node mapping, Crepet et al. (2004) projected an origin for monocots of no later than 98 Ma, consistent with some model-based projections (Magallón & Sanderson, 2002). Despite the projections of an Early Cretaceous origin, the accuracy of the Lower Cretaceous fossil record of monocots (Doyle, 1973) has recently been challenged (Gandolfo et al., 1998, 2000; Crepet et al., 2004), but a recent report of distinctive pollen of Araceae may provide valid Lower Cretaceous evidence of the monocots (Friis et al., 2004). With respect to traditionally cited monocot fossils of Early Cretaceous age, Gandolfo et al. (2000) make the case that the common Early Cretaceous angiosperm fossils, leaves, and pollen are not sufficiently divergent from basal ANITA clade dicots in characters that have been preserved to allow their unequivocal identification as monocot remains. With respect to dispersed palynomorphs, it is not clear that monosulcate pollen with reticulate micromorphology and a gradient in lumina size is restricted to monocots or even to angiosperms (Zavada, 1984).

Monocots from the Cretaceous (Turonian) are represented by what are generally agreed to be flowers representing Triuridaceae (see discussion above; Figs. 2A, B, 10A), a family of saprophytes with very simple and presumably highly reduced vegetative habit. Inasmuch as they nest with modern triurids in phylogenetic analyses (Gandolfo et al., 2002), these fossils suggest that the saprophytic habit had evolved within monocots by the Turonian and, given the presumably derived nature of the saprophytic habit in monocots, are indicative of an earlier origin of monocots consistent with timing suggested by minimum age node mapping and some molecular clock–based models (Bremer, 2000). Monocots are not well represented in progressively younger Cretaceous deposits. Zingiberaceae fruits and leaves are found in Campanian deposits, and Musaceae are known from petrified fruits in the Deccan Intertrappean series (Hickey & Peterson, 1978; Friis et al., 2006). Palms appear in the Campanian, and pollen similar to modern grass pollen appears in the Maastrichtian (Crepet et al., 2004; Friis et al., 2006), suggesting that grasses originated earlier than indicated by the first megafossil remains of leaves and florets/pollen in the Lower Eocene (Fig. 7A, B; Crepet & Feldman, 1991). Furthermore, Prasad et al. (2005) have reported fascinating grass and palm remains from the Deccan Intertrappean series, consistent with reports of Cretaceous palm pollen (Harley, 1996; Harley & Zavada, 2000). The monocot fossil record is better in Tertiary deposits, but some major groups have little or no fossil record (e.g., orchids; Daghian, 1981; Herendeen & Crane, 1995; Gandolfo et al., 2000).

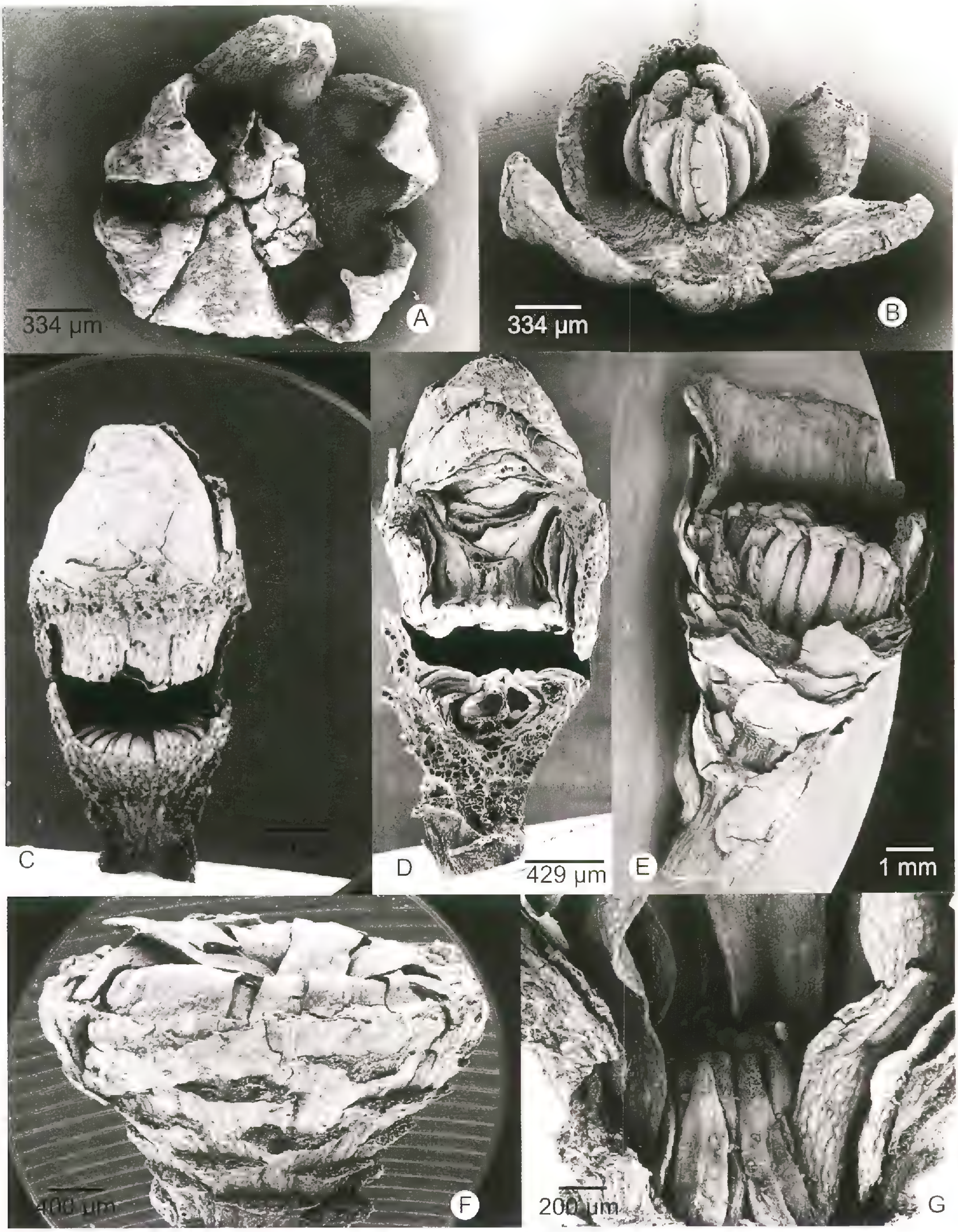

Figure 2. Charcoalified flowers from the Turonian of New Jersey. —A. Cornell University Paleobotanical collection number CUPC1269. —B. Flowers of Triuridaceae showing six tepals and three stamens with connective extensions (CUPC1260). —C, D. *Microvictoria* (Nymphaeaceae) illustrating the morphology and arrangement of floral parts that conform with those of modern *Victoria* Lindl. in the Nymphaeaceae (CUPC1175). —E. *Cronquistiflora* Crepet & Nixon (Magnoliales) illustrating the tepal-bearing floral cup and numerous spirally arranged carpels (CUPC1175). —F, G. *Detrusandra* Crepet & Nixon (Magnoliales) showing floral cup with incurved stamens and staminodes and spirally arranged carpels within (CUPC1188).

MAGNOLIALES

Fossil flowers of apparent magnolialean affinities are a relatively common component of the Sayreville Turonian angiosperm flora. Interestingly, there are a greater number of magnolialean taxa represented by flower fossils in these sediments than by dispersed pollen (Brenner, unpublished palynological data). This observation raises questions about relative rates of character evolution in Magnoliales, suggesting that pollen character evolution lags floral morphology, a phenomenon also observed in other taxa from these Turonian sediments. Thus, the pollen record by itself may not reveal the full extent of Turonian or earlier magnolialean diversity because dispersed grains representing different taxa may have too many common characteristics to be distinguished. This phenomenon also raises questions about the representative nature of the dispersed angiosperm pollen flora in general in the context of overall patterns of angiosperm evolution.

Among putative magnolialean taxa from Sayreville, two, representing extinct lineages, have been described and have a common characteristic: a cupular receptacle that is also shared by numerous undescribed fossils from the same sediments (Crepet & Nixon, 1998a, unpublished data; Fig. 2E–G). These remain the only two representatives of Magnoliales to be identified in phylogenetic context (Fig. 2E–G) and, while clearly extinct taxa, suggest a minimum age for the Magnoliales of 98 Ma using minimum age node mapping consistent with the compression fossils reported by Crane and Dilcher from Kansas in the U.S.A. However, these have not been verified in phylogenetic context (Dilcher & Crane, 1984; Crane & Dilcher, 1984; Figs. 1C, D, 8B, C). Very interesting new discoveries of entire compressed plants from Brazilian Aptian–Albian deposits have been compared with Magnoliales, but assessments of their affinities have been based on comparisons of characters arranged in tables, a methodology discussed above that suffers from being, essentially, a phenetic approach to identification with inevitable subjective elements (Mohr & Eklund, 2003). The possible weaknesses of this mode of identification are particularly evident in the instance of these Aptian–Albian magnolialean flowers, where comparisons extended to purported affinities with extant Eupomatiaceae are based on the presence of glandular tufts on what are characterized as staminodes in the context of an additional suite of generalized characters. The affinities of these fossils seem in need of further review, given that modern Eupomatiaceae have a distinctive cupular floral receptacle (in contrast to these fossils) and a number of distinctive features that are either not preserved in these fossils or have not been elucidated by the techniques employed by the authors (Mohr & Eklund, 2003). Thus, the characters that are reported in tables and illustrated there do not seem to convincingly restrict these fossils to the modern taxon Eupomatiaceae.

Generalized Cretaceous angiosperms including these putative Magnoliales are a particularly good example of a critical group of available fossils that should be carefully analyzed in phylogenetic context because it is among this general class of fossils (i.e., Early Cretaceous angiosperms with conduplicate carpels) that valuable insights might be gained into early angiosperm radiation. However, due to their many common features, full understanding of the pattern of evolution of these taxa and its implications is not likely to be gained from uncritical assessments of affinities that do not involve phylogenetic analyses and that tend toward forcing extinct taxa into extant taxonomic categories, thus potentially obscuring information that might otherwise reveal evolutionarily significant patterns. This is an area that needs attention, but the fossil record suggests that the Magnoliales will be older than the conservative estimate provided by Crepet et al. (2004).

CALYCANTHACEAE

The earliest taxon assignable to the Calycanthaceae based on identification in phylogenetic context is *Jerseyanthus* Crepet, Nixon & Gandolfo (Figs. 3A, B, 9A, B; Crepet et al., 2004). This taxon is distinguished by unusual staminodes or "tepals" (Fig. 3A, B; Staedler et al., 2007) and remarkable branched-stamen connective extensions that were discovered in the fossil before reports of less elaborate but similar connective extensions in modern taxa (Staedler et al., 2007). However, there is an earlier report of a fossil member of the Calycanthaceae, *Virginianthus* Friis, Ekland, Pedersen & Crane (Friis et al., 1994), but this fossil taxon is more generalized than modern calycanthoid taxa and, among other things, has monosulcate pollen consistent with a trend in pollen morphology evolutionarily lagging behind floral morphology that has been observed in other taxa discussed in this article. Thus, the presence of more generalized pollen does not necessarily remove this taxon from Calycanthaceae, but, upon phylogenetic analysis including all of its characters, *Virginianthus* appears in an unresolved position basal to the remaining Laurales or as sister to the entire Laurales excluding the Calycanthaceae, while *Jerseyanthus* nests with the monophyletic Calycanthaceae (Crepet et al., 2004).

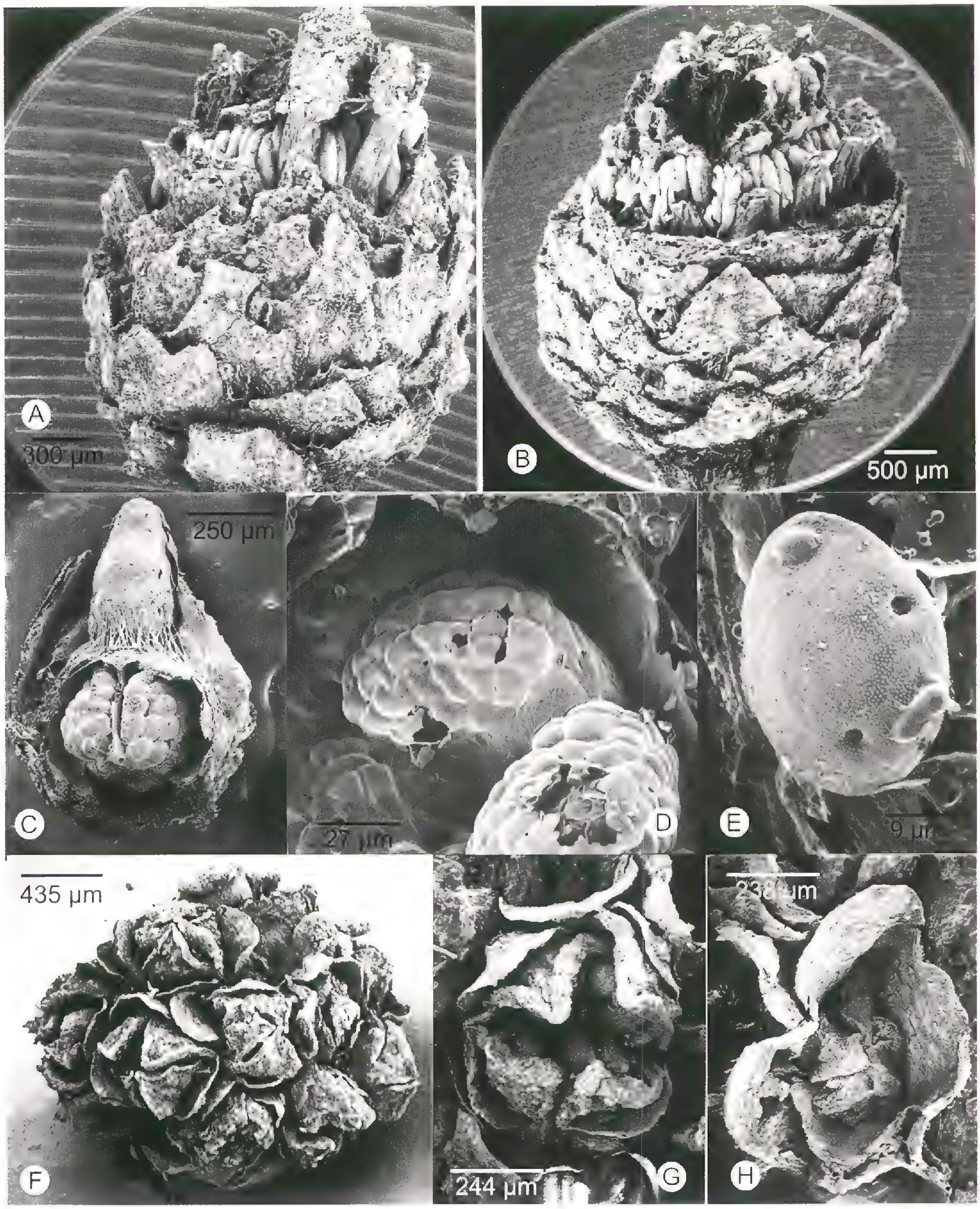

Figure 3. Charcoalified flowers from the Turonian of New Jersey. —A, B. *Jerseyanthus* (Calycanthaceae) showing stalked staminodes (A) and elaborate connective extensions (B) (CUPC1484). C–E. Flower sharing basic characters with modern Caryophyllales showing well-preserved ovules within the ovary (CUPC1507). —D. A single well-preserved ovule showing its campylotropous nature. —E. A pantoporate pollen grain from one of the anthers. F–H. A hamamelidaceous staminate inflorescence showing stamens within a tepal cup (F, G). —H. A floret with stamens removed to illustrate the position of the staminodes (CUPC1160).

CARYOPHYLLALES

The Caryophyllales have only been reported once from Cretaceous deposits (Nixon & Crepet, 1993b). These Turonian fossil flowers may represent this order based on very distinctive pantoporate pollen and generally similar floral morphology (Figs. 3C–E, 9C, D), but there are differences, notably partitions retained in the ovary, but these could be the result of the fossils having been preserved at a relatively early

developmental stage, consistent with the early developmental stages of the well-preserved campylotropous ovules (Fig 3D; Nixon & Crepet, 1993b). Although they possess significant synapomorphies that define the caryophylls, these fossils have not been fully analyzed in phylogenetic context and these results are preliminary.

CORE EUDICOTS, SAXIFRAGALES, HAMAMELIDACEAE

Exceptionally preserved staminate and pistillate inflorescences and detached stamens have been discovered from Turonian deposits at the Crossman site in New Jersey, U.S.A. (ca. 88.5–90.4 Ma) in the Raritan Formation of New Jersey (Crepet et al., 1992; Fig. 3F–H). The fossils have a combination of floral and pollen characters found in various genera of modern entomophilous and anemophilous Hamamelidaceae and anemophilous *Platanus* L. (Platanaceae), including a sepal cup, staminal tube, and apparently nectariferous staminodes (these characters are consistent with insect pollination). This unnamed fossil taxon (Fig. 3F–H), while grouping with modern Hamamelidaceae in phylogenetic analysis, is an example of a mosaic of familial-level characters relative to modern taxa and might have been hard to place outside of phylogenetic context (i.e., the character table comparison method would result in subjective assignment). Interestingly, these fossils have apparent staminodal nectaries (based on position and structure) with morphology intermediate between the fossils' functional stamens and modern hamamelidaceous petals (Fig. 3H). They are positioned within the fossil flowers where petals are found in modern related genera (Fig. 3H), suggesting the possibility that petals originated from such staminodes in the hamamelid lineage. The appearance of the character complex embodied in these flowers during the late Middle Cretaceous may signal the early stages of the relationship between specialized pollinators, such as bees, and the hamamelid–rosid–asterid lineage of angiosperms, arguably one of the most important events in angiosperm radiation (Crepet et al., 1991; Crepet, 1996) and one reflected in the contemporary record of other angiosperms (Figs. 12, 13).

CORE EUDICOTS, SAXIFRAGALES, ALTINGIACEAE

Zhou et al. (2001) described fossil capitate inflorescences that have perigynous florets with a hypanthium, two-carpellate gynoecia, unisexual flowers, phyllomes, numerous ovules per carpel, a three-layered carpel wall, and tricolpate reticulate pollen (Fig. 4A, B). Vessels in the inflorescence pedicel have scalariform perforation plates with oblique end walls and scalariform and opposite/alternate intervascular pitting (Zhou et al., 2001). As in the case of the fossil hamamelidaceous inflorescences described above, this fossil taxon includes a mixture of characters relative to the sets found in modern taxa within Hamamelidaceae, but cladistic analysis suggests that these fossils represent a basal member of the Altingiaceae that lacks the derived pollen found in extant Altingiaceae and retains the more plesiomorphic tricolpate pollen found in most of the Hamamelidaceae. This phenomenon, i.e., generalized pollen relative to morphology of the flower and inflorescences, is observed in other fossil angiosperm taxa from these Turonian sediments (including the Iteaceae; Hermsen et al., 2003). Also, as in the case of the hamamelidaceous taxon above, certain characters of the fossil including phyllomes with stomata, short and straight styles, and small perprolate pollen grains also indicate the possibility of insect pollination.

CORE EUDICOTS, SAXIFRAGALES, ITEACEAE

Fusainized three-dimensionally preserved flowers from the Turonian Raritan Formation of New Jersey (*Divisestylus brevistamineus* Hermsen, Gandolfo, Nixon & Crepet and *D. longistamineus* Hermsen, Gandolfo, Nixon & Crepet) provide another example of fossil taxa whose character complements are not found in any single extant family (Figs. 4C–E, 10C, D). In cases of this nature, tabular comparisons might lead to subjective assignments of affinities through some sort of intuitive character weighting, whereas inclusion of their characters in phylogenetic analysis provides an objective and transparent method for assessing their relationships. These are the kinds of analyses that also reveal trends in character evolution within monophyletic groups. These fossil species (*D. brevistamineus* and *D. longistamineus*) share characters with both Saxifragaceae and Iteaceae in the order Saxifragales. Similarities include a pentamerous perianth, calyx fused below into a hypanthium with free sepal lobes above, haplostemonous androecium with stamens situated opposite the calyx lobes, inferior ovary, bicarpellate gynoecium, numerous ovules on axile placentas, conspicuous intrastaminal nectary ring, and capsulate fruits that open apically. Distinctively, carpels and stigmas are fused, while the styles are free, indicating closer affinities with extant Iteaceae, whereas other characters, such as basifixed anthers in *D. brevistamineus*, tricolpate and striate pollen grains, and anomocytic stomata, indicate closer affinities to Saxifragaceae. Cladistic analyses using molecular and morphological characters, and morphological characters only (Hermsen et al., 2003), however, demonstrate that these

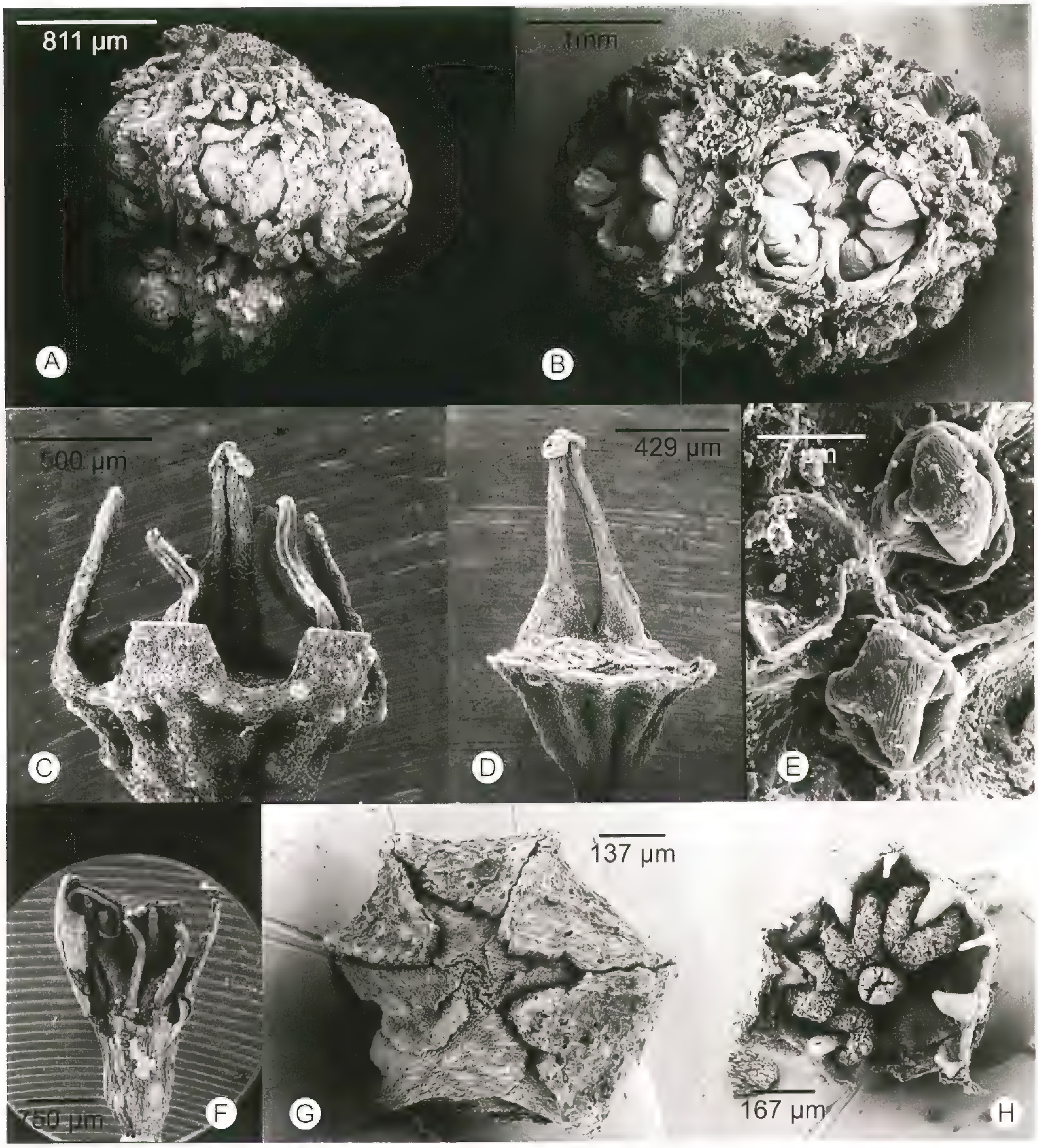

Figure 4. Charcoalified flowers from the Turonian of New Jersey. —A–B. *Microaltingia* Zhou, Crepet & Nixon (Altingiaceae) with bicarpellate florets, phyllomes, and numerous ovules/carpel (A, CUPC1064; B, CUPC1063). C–E. *Divisestylus longistaminous* (Iteaceae). Elongate distally fused styles are distinctive characters of this taxon. —C. Sepals and elongate stamens (CUPC1349). —D. Fusion at the stigmas (CUPC1359). —E. Tricolporate pollen on an anther (CUPC1359). –F. Myrtalean flower with an elongate floral tube, two incurved styles, and coiled stamen filaments (CUPC1510). —G, H. Myrtaceae, hexamerous flower with contorted corolla lobes, phyllomes alternating with stamens, and hexamerous, epigynous ovary (CUPC1506).

fossils share a more recent common ancestor with Iteaceae than Saxifragaceae, thereby making *Divisestylus* Hermsen, Gandolfo, Nixon & Crepet the oldest fossil taxon known with clear affinities to Iteaceae. These fossil taxa are distinguished by having tricolporate pollen (Fig. 4E), a more generalized pollen aperturial configuration than the dicolporate pollen found in modern species of Iteaceae.

ROSIDS, MYRTALES

Myrtaceae are not definitively represented by fossil evidence until approximately 65 Ma (Crepet et al., 2004); however, Turonian fossils with a hypanthium, elongate floral tubes, and coiled stamen filaments may be examples of the Myrtaceae (Fig. 4F). These fossils require further study and, despite their apparent

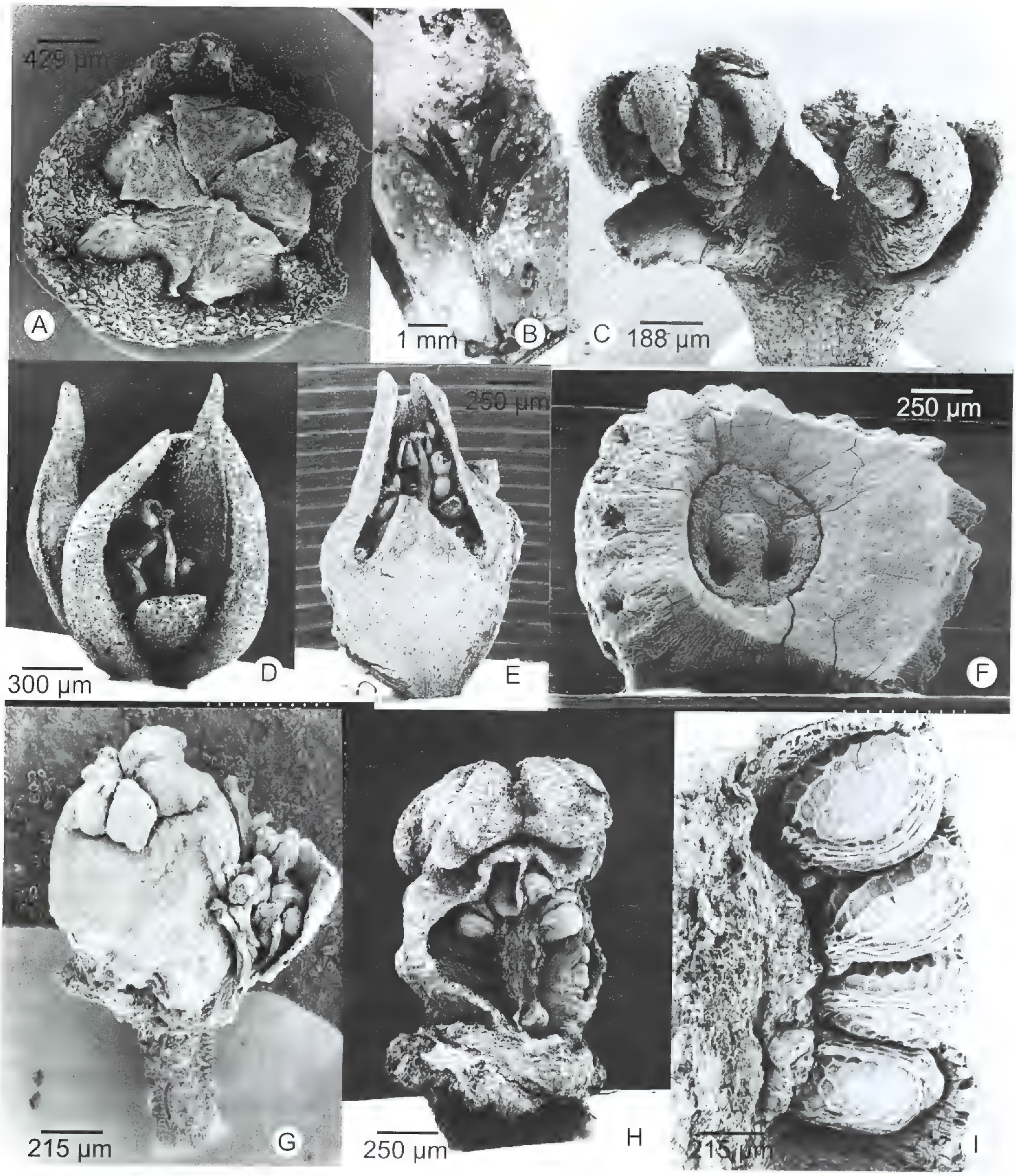

Figure 5. —A. *Astronia cumingiana* (BH79113) in modern Myrtaceae. —B. Fossil fagalean inflorescence preserved in amber from the Turonian of New Jersey. C–I. Charcoalified flowers from the Turonian of New Jersey. —C. Part of a robust inflorescence bearing bisexual fagalean florets similar to those illustrated as seen in Fig. 5E. —D. Staminate fagalean floret. —E. Bisexual fagalean floret. —F. Fruit of Juglandaceae. G–I. *Paleoclusia*. —G. Flower with cuneiform stigmas and fascicled stamens. —H. Pistil with broken ovary wall revealing anatropous ovules. —I. Mature seeds within the ovary.

possession of certain synapomorphies, phylogenetic placement to confirm apparent affinities.

Well-preserved flowers having a set of characters typical of less-specialized Melastomataceae (e.g., as in *Astronium* Jacq.; Fig. 5A) are also found in Turonian deposits (Figs. 4G, H, 5A, 10E, F). These fossils, if their affinities are confirmed, may resolve disputes about a possible Gondwanan origin of melastomes by revealing that they originated early enough so that complex long-distance dispersal models are not needed to explain their modern-day distribution (Raven & Axelrod, 1974; Renner et al., 2001; Morley & Dick, 2003).

EUROSIDS I, FAGALES

Flowers, inflorescences, and pollen (Figs. 5C–E, 11A, B) and another taxon represented by flowers in amber (Fig. 5B) suggest Fagales were extant by the Turonian (Nixon & Crepet, 1994). Charcoalified mesofossils have six tepals in two whorls and are either staminate (Figs. 5D, 11A, B), or bisexual with a well-developed ovary (Figs. 5E, 11B). They are linked by similar floral structure and identical pollen. There are six stamens in each type of flower, and in staminate florets, stamens consist of long filaments and dorsifixed anthers bearing tricolporate pollen with exine micromorphology similar to that of modern castaneoids and generalized Saxifragales. Stamen filaments are flanked by globose structures bearing trichomes that may be part of a nectary disc or represent separate nectaries (Fig. 11A). Ovaries are tricarpellate trigonous and slightly winged as in fruits of certain modern Fagaceae (Figs. 5E, 11B). There are three recurved styles with adaxial grooves (Fig. 11B). Characters of flowers in conjunction with compact, apparently fleshy bisexual flower-bearing inflorescences (Fig. 5C) suggest a morphocline leading to the more compact inflorescences observed in more recent Fagales (Nixon & Crepet, 1994; Sims et al., 1998) and, ultimately, to cupules characteristic of modern Fagales and are indicative of fagalean affinities that have been preliminarily confirmed by phylogenetic analyses (Gandolfo et al., in prep.). The floral characters also suggest insect pollination because the combination of well-developed tepals, nectaries, and pollen of a size incompatible with wind dispersal (Whitehead, 1969) is indicative of insect pollination. Insect pollination in ancestral Fagales would also be compatible with apparent insect pollination in early Platanaceae as suggested by Friis et al. (1988) and also with floral characters in early Juglandales that also are consistent with insect pollination, suggesting that extant wind-pollinated hamamelids are derived with respect to pollination biology.

EUROSIDS I, JUGLANDALES

Excellently preserved fossils of Juglandales from Santonian Coniacean deposits have provided the first important insights into the affinities of the *Normapolles*-bearing taxa and the history of the Juglandales (e.g., Friis, 1983; Sims et al., 1999; Schönenberger et al., 2001) and have provided further insights into pollination biology of early Juglandales. Several inflorescence fragments with associated *Normapolles* pollen and nuts (Nixon, in prep.; Fig. 5F) suggest Juglandales were present by the Turonian.

EUROSIDS I, MALPIGHIALES, CLUSIACEAE

Remarkably well-preserved flowers are five-merous with fascicled stamens and cuneiform stigmas and are almost identical to flowers of modern Clusiaceae in every respect down to pollen morphology and resin canals in the floral tissues (Fig. 5G–I). These flowers group with modern Clusiaceae in phylogenetic analysis (Crepet & Nixon, 1998b).

EUROSIDS II, BRASSICALES (CAPPARALES)

A distinctive Turonian taxon is represented by flowers having a combination of characters now found in families in the order Capparales (now included in Brassicales; Angiosperm Phylogeny Group, 2003). This taxon, *Dressiantha* Gandolfo, Nixon & Crepet (Gandolfo et al., 1998b), has flowers with a well-developed gynophore, a distinctive sepal arrangement, an unequal petal size (bilaterality), monothecal anthers, and a bicarpellate gynoecium, characters found in extant families in the order Brassicales (Angiosperm Phylogeny Group, 2003; Fig. 6D–F). This taxon represents the oldest known fossil record for the Brassicales and has been important in dating in the history of the related extant taxon *Arabidopsis* (DC.) Heynh. Interestingly, in a recent paper, de Craene and Haston (2006) place the fossil in a different morphology matrix from the one used by Gandolfo et al. (1998b), who used the Rodman (1991a, b) matrix. The de Craene and Haston matrix was larger and included characters, such as wood anatomical features, not found in the *Dressiantha* fossil. It may be that the resultant addition of missing values for *Dressiantha* in their matrix might account for the anomalous change in the position of this fossil taxon from Brassicales to Sapindales. The change is anomalous because, as discussed by de Craene and Haston (2006), there is no conflict between morphological characters found in Brassicales and those displayed by the fossil flowers. This is a good example of the value of the medium of phylogenetic context in discussing affinities of fossil taxa. Different outcomes can be objectively analyzed and explained; new questions can be posed in a level of discourse that is both appropriate and potentially illuminating.

ASTERIDS, ERICALES OR ERICANAE, THEACEAE

Flowers five-merous with three carpels, fascicled stamens, and tricolporate pollen represent Theales, are diverse in the Turonian deposits of northern New Jersey (Figs. 6A, 11C, D; Crepet et al., 1996), and are also represented in somewhat younger (Campanian) deposits in Georgia (Keller et al., 1996).

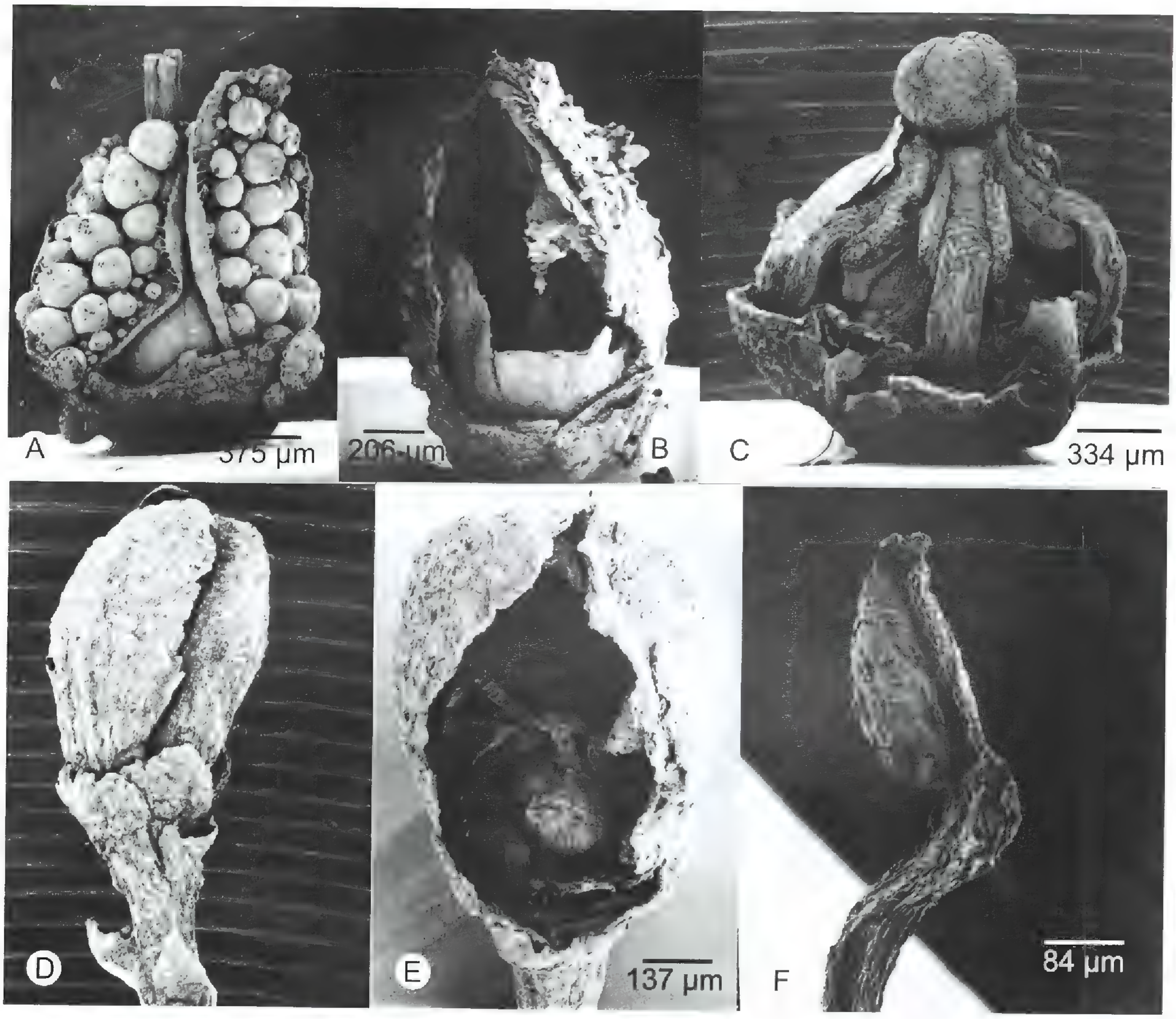

Figure 6. Charcoalified flowers from the Turonian of New Jersey. —A. A Thealean flower with remarkable abaxial sepal glands. —B. *Paleoenkianthus* Nixon & Crepet (Ericaceae) flower bud broken to show the corolla, pistil, and stamens within. —C. An ericalean flower with stamen tubules. D–F. *Dressiantha* (Brassicales). —D. Flower illustrating the bilaterally symmetrical corolla in bud. —E. An open flower bud showing the pistil and parts of stamens. —F. A single stamen illustrating the geniculate filament.

ASTERIDS, ERICALES OR ERICANAE, ERICACEAE

Fossils of Ericanae are interesting and appear first in Turonian deposits (Figs. 6B, C, 11E, F) and shortly thereafter in Santonian–Campanian deposits of Sweden (Friis, 1985; Schönenberger & Friis, 2001). They are remarkably diverse in the Turonian and demonstrate a complex of characters that is now associated with highly specific, usually apid pollinators. Among these, a more general and basal Ericalean taxon has elongate sepals, nectary disc, carpels with separate stigmas, a sympetalous corolla, and two whorls of stamens with stamen awns (Nixon & Crepet, 1993a). A more interesting association of closely related, more derived ericalean taxa is linked by a basic five-merous floral plan and a set of shared but variable characters (Figs. 6C, 11E, F). These include abaxial sepal glands, sepal indumentum, haplostemony, dorsifixed anthers, basic pollen morphology, flattened stamen filaments, staminodal nectaries, and superior ovaries with five-lobed stigmas having distinctive circular indentations at the junctures of the lobes. However, there are distinct variants representing at least three taxa and probably more. One taxon has anthers without appendages or tubules, elongate stamen filaments, monadinous pollen grains, a non-globose stigma, and fruits that are loculicidal capsules. This taxon is the best known among this complex because flowers are occasionally preserved in inflorescences (Fig. 11E). Flowers are subtended by two bracteoles in the axil of foliar bracts (Fig. 11E). Leaves associated with these fossils are folded, with marginal glands similar to those found on the margins of the sepals of flowers representing this taxon (Fig. 11E, F).

A second taxon differs from the first ericad of this five-merous complex in having differently distributed sepal glands, shorter stamen filaments (anthers do not extend beyond the stigma height), stamen filaments with distinctive stellate trichomes that are also found on the pistil, anthers that are notched and have tubules (Fig. 6C), and pollen that is apparently in loose polyads connected by short viscin threads. Both fossil taxa group within Ericaceae upon phylogenetic analysis (Crepet et al., 2004).

Overview of the Angiosperm Fossil Record in the Aptian–Turonian Interval

There is a striking pattern in the fossil records of both angiosperm taxa and their defining floral characters within this interval that has a number of interesting and possibly meaningful implications. Early Cretaceous taxa have a relatively sparse but significant record that has already revealed the timing of the occurrences of major events in angiosperm history including the possible origin of the angiosperms, the evolutionary assembly of the bisexual flower, the origin of the floral envelope, and the origins of several major angiosperm lineages, including the eudicots, accompanied by a corresponding diversification of pollen types. In addition, Early Cretaceous angiosperm fossils include representatives of what appear to be extinct taxa basal to existing lineages such as Nymphaeaceae. Existing evidence indicates that Early Cretaceous taxa included the ANITA clade + Chloranthaceae + the early tricolpate lineages: platanoids and Buxaceae (Drinnan et al., 1991; Pedersen et al., 1991, 1994; Friis et al., 2006). The modern-day diversity of taxa that first appear in the Early Cretaceous is relatively low, with little indication from known fossils that they might have once been more speciose (Magallón et al., 1999; Crepet et al., 2004; Friis et al., 2006). With respect to function, fossil flowers of this time were either structurally appropriate for coleopteran pollinators or suggestive of generalized kinds of insect pollination that may have involved Coleoptera, Diptera, early Lepidoptera (Micropterigidae), and, conceivably, even hymenopteran lineages transitional to Apidae (Thein, 1980; Grimaldi, 1999; Grimaldi & Engel, 2005). The uniformly small floral fossils of this age suggest that their pollinators at least included very small insects (Friis et al., 2006). The possibility that some of these taxa may have been wind pollinated has been raised by Dilcher (2000).

The lower Late Cretaceous time frame (more or less the middle of the Cretaceous), including the Cenomanian, Turonian, and Coniacean ages, is important because angiosperms became dominant in species numbers (inferred from modern-day diversity in clades that appear by that time) during this interval (Fig. 12) and because there was a dramatic coincidence of co-occurring factors that may be significant in explaining some measure of the success of the flowering plants. These include: (1) The striking peak in diversity of angiosperm taxa. There are ca. 39,000 species today included in the dicot taxa known to have existed by that time. Although the early record of the monocots is too fragmentary to give an accurate idea of actual monocot diversity during this Middle Cretaceous interval, they were almost certainly present in greater numbers than indicated by the known fossil floral record, and, thus, total angiosperm species diversity was likely to have been even greater than reliable evidence now indicates. (2) The earliest (Cenomanian) appearance of the first cyclic eudicot flower that had both petals and sepals in addition to definite numbers of floral parts (Figs. 1E, F, 8D; Basinger & Dilcher, 1984). Also note that cyclic floral parts in other configurations are considered widespread if not fundamental to most angiosperm taxa by some authors (e.g., Zanis et al., 2003). (3) The dramatic Turonian appearances of a wide variety of floral morphologies, some now specifically associated with more derived pollinators including hymenopterans (Fig. 13). The evolution of some of the latter floral types (e.g., those having a fused corolla or bilateral symmetry, etc.) would logically have depended on the prior occurrence of the relatively simple cyclic flower because these more specialized configurations would not have been attainable in a single step from ancestors with a more generalized floral plan that included at least some multiple spirally arranged floral parts (Endress, 2001). Given the timing observed in the fossil record (i.e., the rapid appearance of derived floral configurations closely following the appearance of the cyclic flower with regular numbers of parts), various floral specializations for more derived pollinators must have evolved rapidly in the Cenomanian–Turonian interval, indicating possibly rapid adaptation to radiating hymenopteran or other highly derived pollinators. This possibility is consistent with the first appearances in the Turonian of taxa that are now specifically and closely associated with particular and derived Apidae (e.g., the Clusiaceae, *Paleoclusia* Crepet & Nixon, with Meliponini). In addition, this fossil Clusiaceae indicates that even at this relatively early stage of their history, at least some of these fossil taxa apparently had the same reward structure as their modern relatives (in this instance, resins; Crepet & Nixon, 1998b). (4) Timing of the early fossil record of bees. The observed Middle Cretaceous burst of angiosperm species diversity is followed relatively closely by the first fossil evidence

of bees. The earliest bees are preserved in Upper Albian Burmese amber (Poinar & Danforth, 2006), and another fossil bee that shares many characters with modern Meliponinae is preserved in apparently Upper Cretaceous amber of uncertain age (Grimaldi, 1999; Engel, 2000), but the possibility that this fossil from New Jersey is of Turonian or even earlier in age is supported by the presence of the fossil *Paleoclusia* noted above from Turonian deposits of New Jersey, U.S.A., with similar morphology and the same apparent reward system that today ties the Meliponinae to the Clusiaceae (Crepet & Nixon, 1998b).

Post-Turonian Cretaceous Deposits

The post-Turonian pre-Maastrichtian fossil record of angiosperms is not as complete as that of the record in the Cenomanian–Turonian interval and, so far, has failed to provide a corresponding level of detail of subsequent Late Cretaceous angiosperm radiation. As it is presently understood, the fossil record documents a limited (but significant) number of innovations between the Turonian and Maastrichtian. This interval is characterized by the radiation of rosid orders Fagales and Juglandales that appear first in the Turonian, represented by flowers, partial inflorescences, and fruits (Fig. 5F; Sims et al., 1998). Inferences from other carefully analyzed plant organs, while perhaps risky without more detailed knowledge of the relative rates of evolution among different plant organs, do suggest that monocot diversification proceeded throughout the Upper Cretaceous with evidence of Zingiberales (fruits and leaves) and palms (fruits, pollen, and leaves) in this interval (Hickey & Peterson, 1978; Daghlian, 1981; Herendeen & Crane, 1995; Gandolfo et al., 2000). Maastrictian floral remains are generally not preserved in great detail but are significant because the record of monocots suggests that they had radiated considerably by this time, consistent with the occurrence of Triuridaceae in the Turonian and other Lower Cretaceous remains that have been attributed to monocots. Finally, as noted above, pollen with morphological and structural features compatible with the distinctive pollen of grasses is known from Maastrichtian deposits (Linder, 1986; Crepet & Feldman, 1991), and grass-like leaf remains have been isolated from Maastrichtian dinosaur coprolites (Prasad et al., 2005), further suggesting that monocots had radiated relatively invisibly during much of the Cretaceous. Interestingly, there is not a great deal of evidence in the pre-Maastrichtian–Late Cretaceous of taxa with the kinds of fruits that are adapted for animal seed dispersal in groups that now are characterized by such dispersal mechanisms (e.g., some Juglandaceae and Fagales).

Early Tertiary–Paleogene

There is a burst in Tertiary angiosperm diversification characterized by the radiation of grasses, radiation of Fagaceae and Juglandaceae, appearance and radiation of Euphorbiaceae (Crepet & Daghlian, 1982, unpublished data), legumes (mimosoid, caesalpiniod, and papilionoid, Fig. 7C–E; Herendeen & Crane, 1992; Lavin et al., 2005), and many asterids in the Asterid I clade (although the fossil record of reliably identified asterids is relatively sparse; Crepet et al., 2004; Friis et al., 2006). This interval represents a period of modernization for many taxa and is also characterized by the radiation of many pollinating insects including the Macrolepidoptera. The Tertiary record of bees is relatively good (Grimaldi, 1999; Grimaldi & Engel, 2005). Perhaps the most dramatic innovations in the Tertiary include the brush flower/inflorescence (mimosoid legumes), the papilionoid legume flower with its pronounced zygomorphy, and the prolonged corolla tube (Fig. 7C–F). In addition, the Tertiary is characterized by the radiation of additional taxa (e.g., asterids and grasses), which include modern relatives with herbaceous habits.

Discussion

There are a number of central questions surrounding angiosperm history in the context of updated fossil evidence.

Angiosperm Origin and Early Radiation

Additional clarification of the structures of early fossil angiosperms and analysis of their relationships in phylogenetic context promise to illuminate early angiosperm history and perhaps to reveal certain important homologies, and already pose certain questions. For example, were early angiosperms apetalous? Are sepals de novo organs, are they modified stamens, or do they have multiple origins and homologues? Did the flower originate by coalescence of reproductive structures borne on elongate axes such as those of *Archaefructus*, and do a number of Early Cretaceous angiosperm flowers represent a morphocline linking *Archaefructus*-like ancestors with condensed flowers, or is this model too simplistic and not supported in phylogenetic context? Such clarifications, if forthcoming and if conducted within a phylogenetic framework, may complement genetic studies of MADS-box genes and transcription factors known to influence floral development in ways that might reveal homologies between angiosperm reproductive structures and those of non-angiosperms (e.g., Theissen et al., 2000).

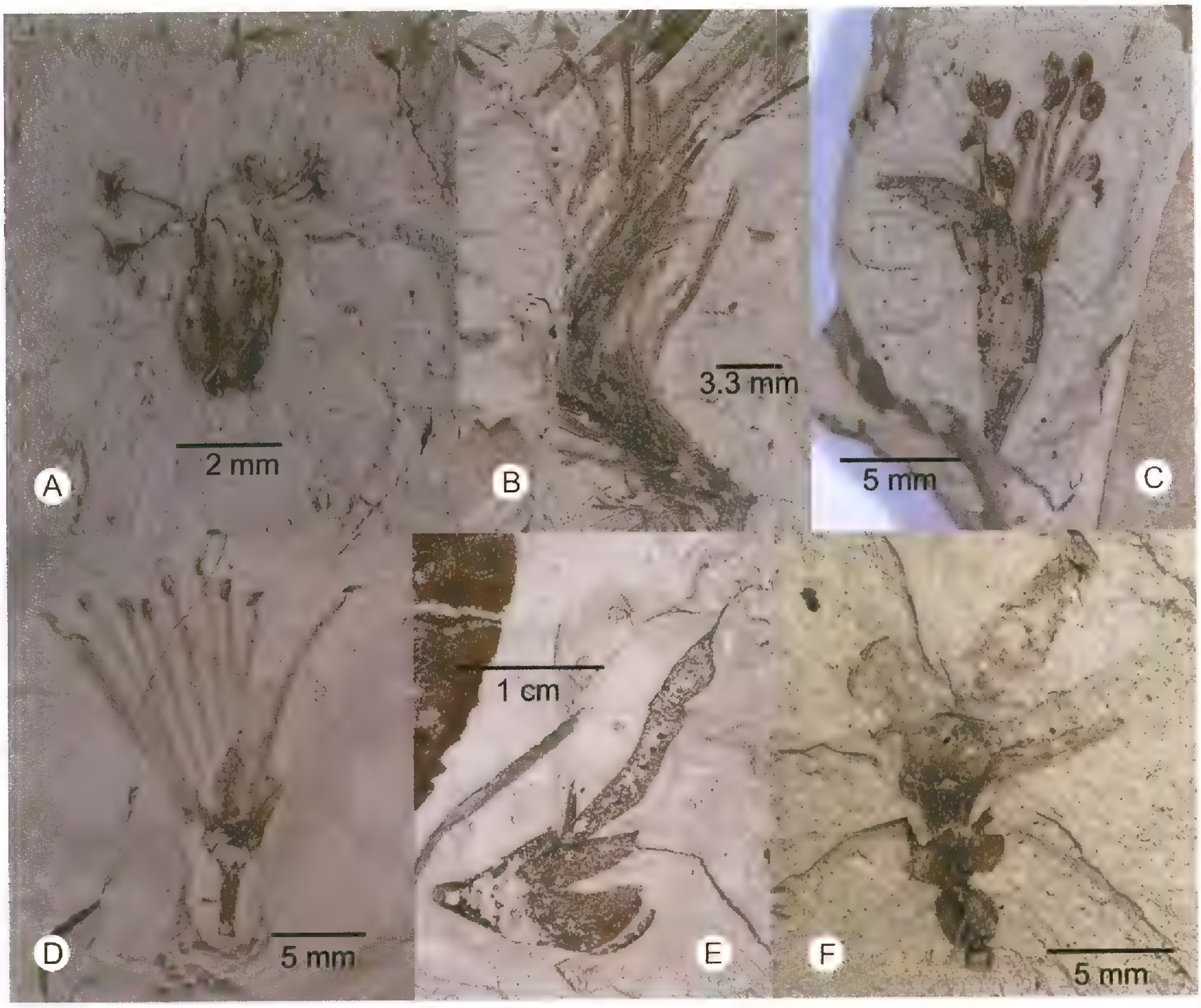

Figure 7. Tertiary compression fossils from the Paleocene–Eocene of Tennessee, U.S.A. —A. Grass spikelet with two florets. —B. Grass rhizome with leaf sheaths. —C. *Protomimosoidea* Crepet & Taylor flower with developing legume. —D. *Protomimosoidea* flower. —E. *Barnabyanthus* Crepet & Herendeen, the earliest papilionoid flower in the fossil record. —F. A flower (Asterid I?) illustrating that the funnelform corolla is well developed at this time.

ANGIOSPERM DOMINANCE IN SPECIES NUMBERS

Given the scale of angiosperm preeminence in modern plant species diversity and its obvious ecological significance, the matter of angiosperm predominance and success has received a great deal of attention and continues to be an area of considerable interest, speculation, and controversy (Raven, 1977; Regal, 1977; Doyle, 1978; Burger, 1981; Stebbins, 1981; Niklas et al., 1983; Crepet, 1984, 1996; Tiffney, 1984, 1986; Eriksson & Bremer, 1992; Waser, 1998; Grimaldi, 1999; Magallón et al., 1999; Verdú, 2002; Feild et al., 2004; Fenster et al., 2004; Grimaldi & Engel, 2005; Friis et al., 2006). There is little doubt that there are separate and possibly complementary reasons for the present level of angiosperm species diversity (Raven, 1977; Crepet, 1984; Tiffney, 1984; Eriksson & Bremer, 1992; Tiffney & Mazer, 1995; Verdú, 2002; Fenster et al., 2004). There have been appraisals of the correlations between diverse angiosperm taxa and derived pollinators (including vertebrates; Raven, 1977), assessments of the potential significance of animal seed dispersers (Tiffney, 1984), and examinations of the possible effects of synergy among multiple factors (e.g., Raven, 1977; Regal, 1977; Crepet, 1984; Tiffney, 1984; Eriksson & Bremer, 1992). In addition, consideration has been given to factors involved in minimizing extinction, as well as to those involved in promoting speciation (Tiffney & Mazer, 1995). There have been challenges to various hypotheses linking angiosperm success to various factors including pollinator specificity and efficiency following the model first proposed by Verne Grant (1949; e.g., Midgely & Bond, 1991; Waser, 1998; Fenster et al., 2004). While there are obvious correlations between hyperdiverse taxa and insect pollinators (e.g., orchids and euglossine bees; Dressler, 1968, 1982), there are obvious alternative attributes or circumstances that may have been responsible for high species diversity

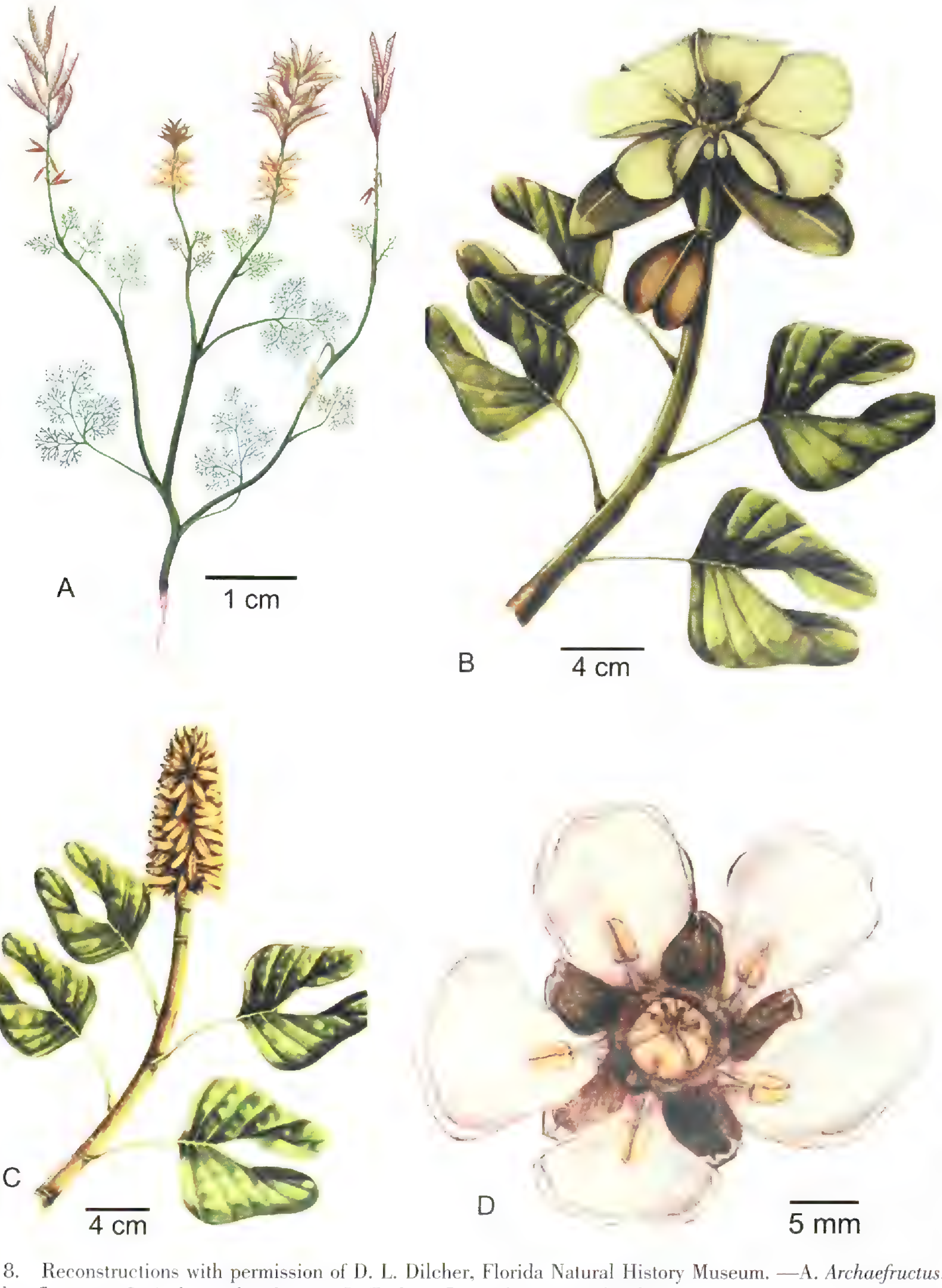

Figure 8. Reconstructions with permission of D. L. Dilcher, Florida Natural History Museum. —A. *Archaefructus*. —B. *Archaeanthus* flower. —C. *Archaeanthus* fruit. —D. Eudicot flower from Rose Creek.

within particular monophyletic groups. Sorting out the relative influences remains very much at issue, and, as molecular genetics studies continue to reveal more about genetic control mechanisms and within-species reproductive barriers (hybrid necrosis; Bomblies & Weigel, 2007), we might reasonably expect the discovery that other factors have had important roles in promoting species diversification in angiosperms.

Currently, our understanding of the fossil record of angiosperms is much improved and more reliable, and we have a better idea of the pattern of appearances of angiosperm families, floral characters, and possible insect pollinators. Likewise, we have this knowledge in the context of a more accurate understanding of overall angiosperm relationships (Soltis et al., 2000), such that some questions concerning angiosperm success can be addressed from a fresh perspective. However, in considering the phenomenon of angiosperm diversification through geologic time, it is important to frame the major ques-

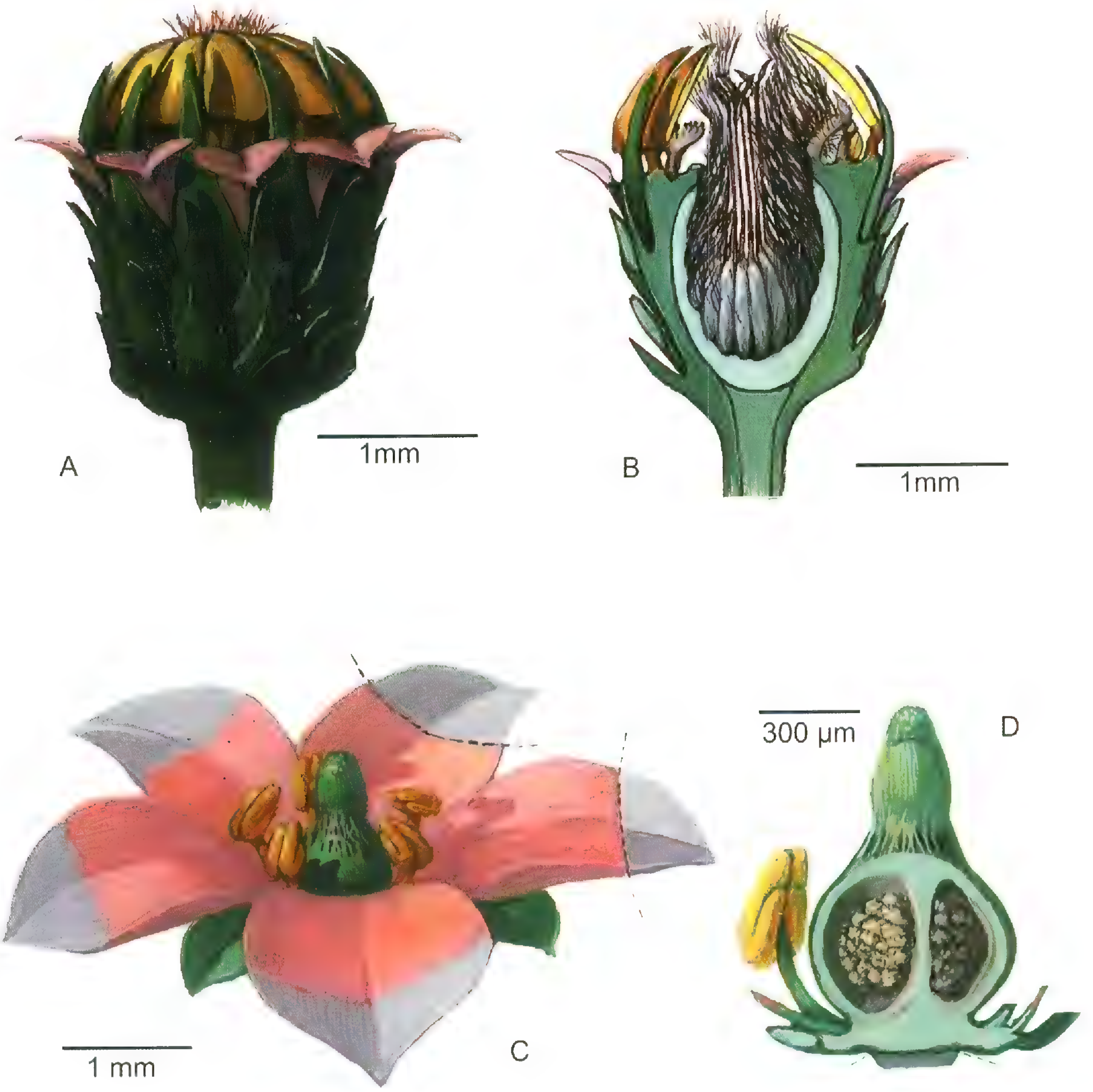

Figure 9. Reconstructions of Turonian fossil flowers with permission of Michael Rothman. —A, B. *Jerseyanthus* (Calycanthaceae). —C, D. ?Caryophyllales.

tions before proceeding to examine how recent, more complete, and reliable evidence affects our possible understanding of them.

With respect to timing, one can ask two questions about angiosperm preeminence in species numbers: (1) When do angiosperms become dominant in species number (measured by first appearances of modern diverse groups), and what are the circumstances that might have been relevant based on timing? (2) How did even greater angiosperm diversity arise following the initial attainment of angiosperm dominance in numbers of plant species?

It is interesting to examine fossil evidence now available with respect to the questions above and to consider whether it rules out any possibilities, such as insect involvement in preeminent angiosperm diversity, due to timing considerations. As noted in the preceding summary of the angiosperm fossil record, conservative estimates of timing in first appearances of angiosperm families suggest that the angiosperms had become dominant by the Turonian (ca. 90 Ma). In fact, families appearing by Turonian times now encompass approximately 39,000 species (Fig. 12), while all gymnosperm and vascular non-seed plant taxa combined today only include about 22,000 species. The dramatic coincidence of characters associated with advanced modes of insect pollination by the Turonian (Fig. 13), the similarity of certain Turonian angiosperm taxa to those now specifically associated with such pollinators, and the proximally temporal fossil evidence of the earliest bees are consistent with insect involvement in the Cenomanian–Turonian proliferation of angiosperm species according to mechanisms discussed by Grant (1949).

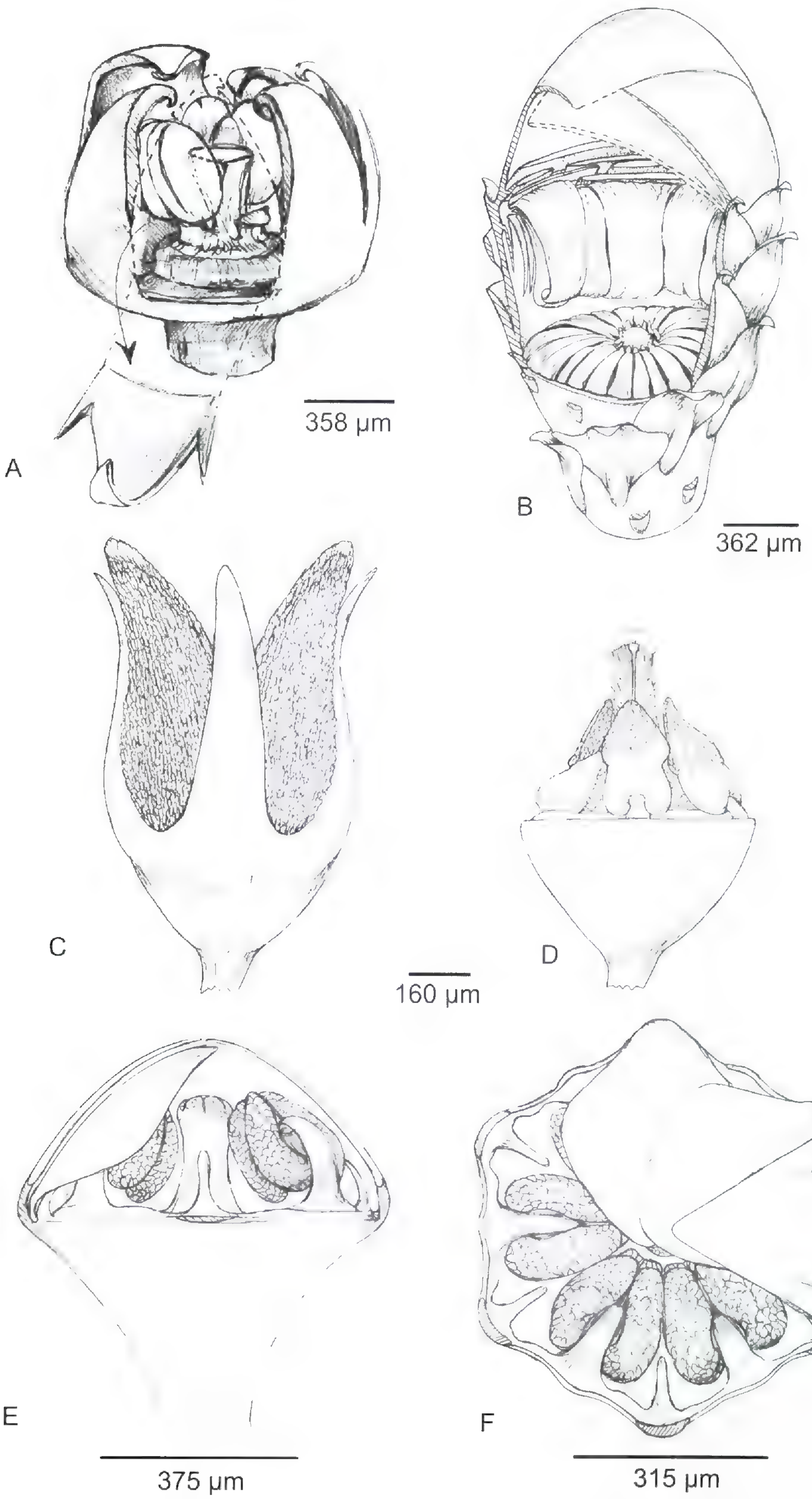

Figure 10. Reconstructions of Turonian fossil flowers with permission of Michael Rothman. —A. *Mabelia* (Triuridacae) illustrating a fossil species with a staminal column, a character also found in modern Triuridaceae. —B. *Microvictoria*. —C. *Divisestylus longistamineus*. —D. *Divisestylus brevistamineus*. —E, F. Reconstructed fossil Melastomataceae.

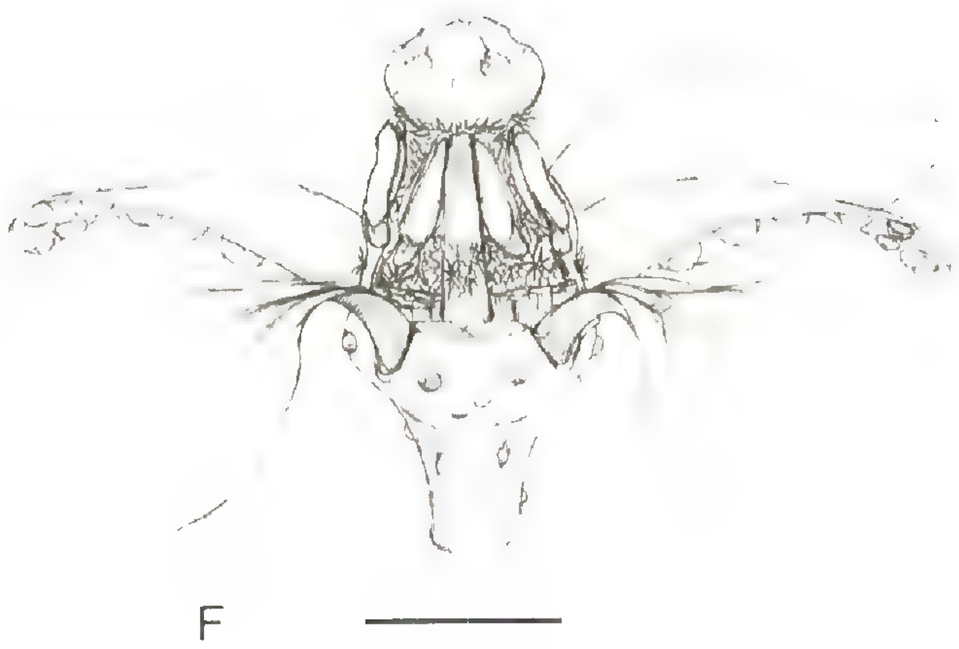

Figure 11. Reconstructions of Turonian fossil flowers with permission of Michael Rothman. A, B. Fagalean flowers. —A. Staminate floret. —B. Bisexual floret (this taxon is andromonoecious). —C, D. A generalized thealean fossil illustrating the pistil with three styles and numerous ovules and the fascicled stamens of uneven length. —E. Ericalean inflorescence with remarkable glands on the margins of the large bracts subtending paired florets, which are themselves enclosed by a pair of bracteoles, each with a terminal gland. —F. Ericalean flower reconstruction.

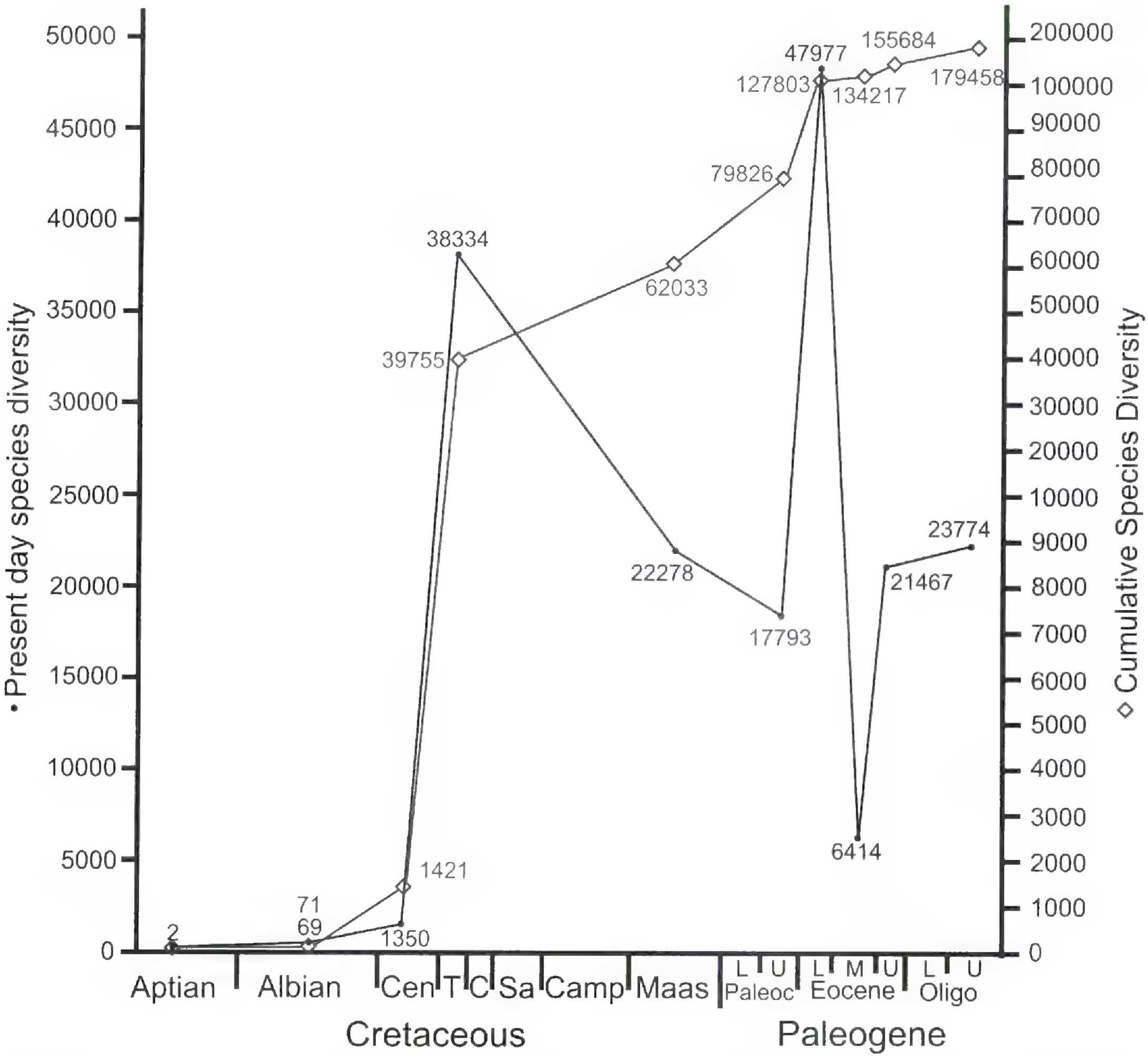

Figure 12. Cretaceous and Paleogene angiosperm species diversity through time. As has been the convention elsewhere, the relative heights of the peaks (black dots) reflect the numbers of species (*y*-axis scale on left) now found in angiosperm families that first occur in the fossil record at the corresponding times indicated on the *x*-axis. Thus, 38,334 species occur in modern families that first appear in the Turonian (see text discussion). Cumulative species diversity through time is illustrated with diamonds, according to the *y*-axis scale on the right side of the figure. Thus, while 38,334 species are now included in families that first appear in Turonian deposits, 39,755 species are now included in all angiosperm families that appeared by the Turonian.

Crepet (1984, 1996), Eriksson and Bremer (1992), Grimaldi (1999), and others. However, based on recent field and related studies, the relationship between pollinators (or classes of them) and speciation is as yet imperfectly understood and in need of further study before a cause-and-effect relationship can be postulated (e.g., Grant, 1994; Waser, 1998; Fenster et al., 2004). Figure 12 also illustrates that a dramatic increase in number of angiosperm species occurs from the end of the Cretaceous to the Middle Eocene. There is a drop in the rate of appearances of new families during the Upper Cretaceous (but against a background of steadily increasing species diversity; Fig. 12), which could be the result of sampling error because there are few, if any, reported fossil localities with preserved diverse fossil flowers between the Coniacean and the Maastrichtian. However, best estimates of timing major groups based on molecular clock models (e.g., Lavin et al., 2005) or on minimum age node mapping (Crepet et al., 2004) suggest this apparent absence of many of the major diverse groups from the Upper Cretaceous implied by fossil evidence is real (Leguminosae, Asterids I, Euphorbiaceae, etc.). Thus, the question arises: What were the factors involved in the proliferation of angiosperm species in the Early Tertiary, and were they different from those involved in the initial establishment of angiosperm species dominance?

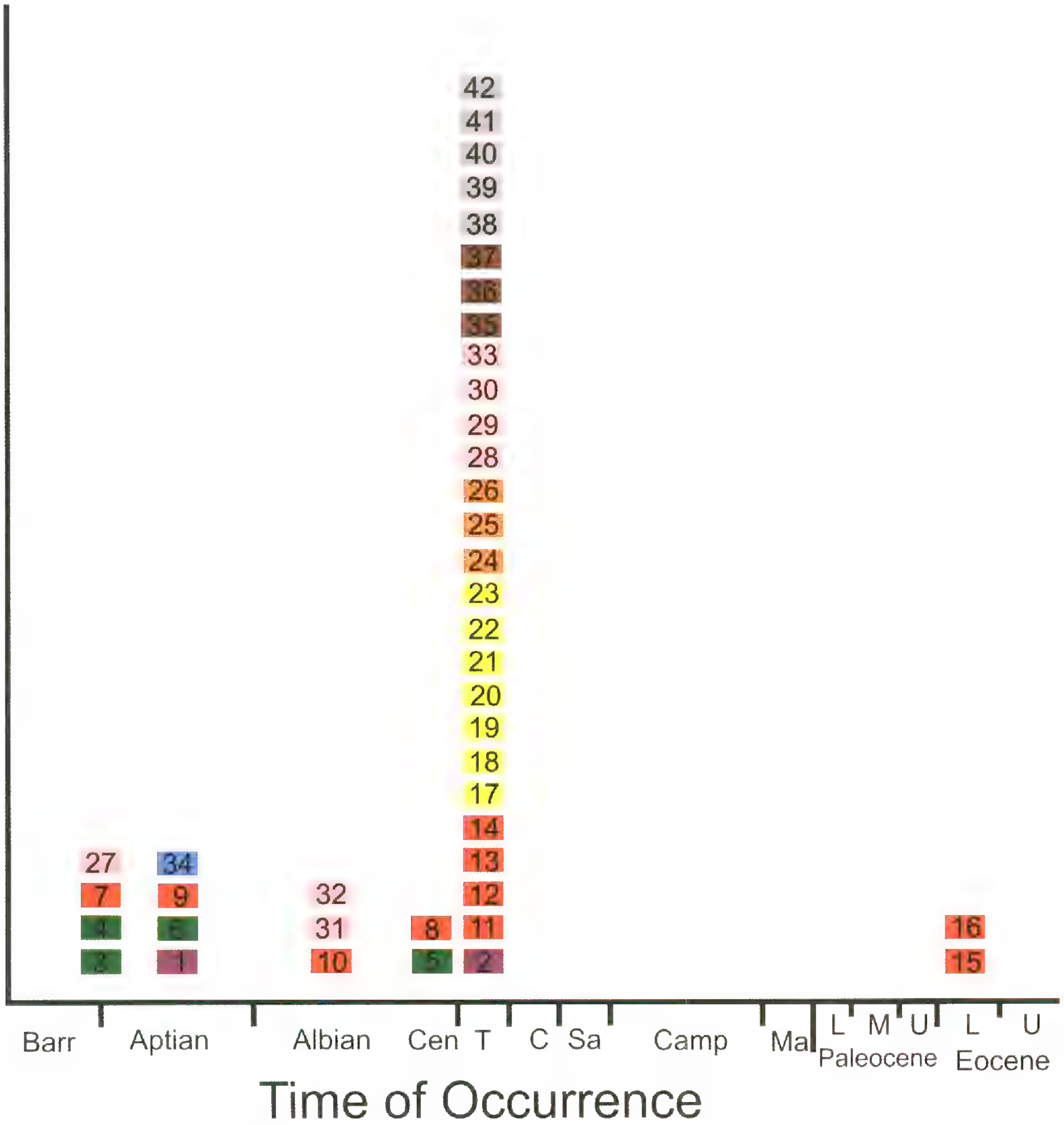

Figure 13. Times of occurrence of select floral characters. These are identified on the figure by numbers in colored boxes corresponding to the character summary below and grouped into color-coded categories (e.g., arrangement of floral parts—green) to illustrate patterns in the evolution of particular categories of floral characters. Times of appearance are noted in the summary below and illustrated on the *x*-axis of the figure. The *y*-axis illustrates numbers of characters occurring at the times indicated on the *x*-axis. **Floral receptacle** (purple). 1. Cupular (Aptian). 2. Hypanthium (Turonian). **Arrangement of floral parts** (green). 3. Paired (Barremian/Aptian). 4. Spiral (Barremian/Aptian). 5. Cyclic (Cenomanian). 6. Coalesced into "flowers" (Aptian). **Floral envelope** (red). 7. Apetaly (Barremian/Aptian). 8. Regular merosity (Cenomanian). 9. Subtending bracts/bracteoles (Aptian). 10. Numerous tepals (Albian). 11. Sepal tube (Turonian). 12. Corolla asymmetry (Turonian). 13. Clawed petals (Turonian). 14. Corolla tube (Turonian). 15. Zygomorphy (Turonian). 16. Prolonged corolla tube (Lower Eocene). **Stamens** (yellow). 17. Fascicled (Turonian). 18. Geniculate (Turonian). 19. With tubules (Turonian). 20. Dorsifixed (Turonian). 21. Inverted (Turonian). 22. Staminal tube (Turonian). 23. Staminodal nectary (Turonian). **Pollen** (orange). 24. Viscin threads (Turonian). 25. Polyads (Turonian). 26. Pantoporate pollen (Turonian). **Gynoecium** (pink). 27. Apocarpy (Barr/Aptian). 28. Inferior ovaries (Turonian). 29. Multiple carpels and elongate style (Turonian). 30. Multiple carpels and single style (Turonian). 31. Carpels fused (Albian). 32. Carpels whorled (Albian). 33. Ovary wall nectary (Turonian). **Sexuality** (blue). 34. Bisexual (perfect) (Aptian). **Reproductive condition** (brown). 35. Monoecy (Turonian). 36. Dioecy (Turonian). 37. Andromonecy (Turonian). **Reward structure** (silver). 38. Nectar (Turonian). 39. Nectary disk (Turonian). 40. Resin (Turonian). 41. Pollen (Turonian). 42. Staminal food bodies (Turonian).

To place the Early Tertiary radiation of angiosperms in the context of earlier events in angiosperm history, early angiosperms originated and diversified in the Lower Cretaceous, enjoying some advantages over contemporary gymnosperms due to their short life cycles, self-incompatibility systems, biosynthetically diverse secondary compounds, probable insect pollination, and possibly wider ecophysiological tolerances

(e.g., Doyle, 1978; Verdú, 2002; Feild et al., 2004) and possibly because of hybrid necrosis (although its phylogenetic distribution in angiosperms has yet to be determined, making it difficult to assess its impact on angiosperm radiation; Bomblies & Weigel, 2007). Toward the Middle Cretaceous, Cenomanian deposits include the first evidence of the regular eudicot flower with distinct corolla and, in evolutionary context, what might be considered developmentally interchangeable, cyclically arranged floral parts. This floral configuration might have constituted a baseline or platform morphology that would facilitate the parsimonious origin of more advanced floral morphologies. Because many families with such derived floral morphologies are now quite diverse, the attainment of such a platform morphology in certain lineages might have catalyzed the quantum leap in angiosperm diversification that closely followed its origin as documented by angiosperms from Turonian deposits. Turonian angiosperm flowers do include those with novel morphologies and characters now associated with bee pollinators (Figs. 5G, 13). These apparently adaptive characters (Fig. 13) also occur in the same geologic timeframe as early bee pollinators, during observed increases in gross angiosperm diversity, and along with first appearances of angiosperm families that now include over 32,000 species. This broad co-occurrence of related factors suggests that the major burst of evolution accomplished in angiosperms by the Turonian supports the possibility that pollinator involvement in numerical species dominance by flowering plants cannot be ruled out based on timing alone. Yet, based on recent studies discussed above, this finding does not determine whether this co-occurrence of factors is a cause of angiosperm success or an effect of it.

Further, with respect to the second question posed above about causes of the later, but equally dramatic radiation of angiosperms in the Early Tertiary, one could ask: if flower-pollinator relationships were critical to angiosperm diversification, why, with the regular cyclic flower, bilaterality, and even the corolla tube established by Turonian times, was there an apparent lag between the Turonian and Lower Tertiary before the resumption of rapid radiation of families that was characterized by even more specific pollinator relationships associated with sometimes even more highly adapted floral structures (Fig. 7C–F)? Here, coordinated character (s.l.) evolution may make sense out of the patterns observed in the angiosperm fossil record. For example, with respect to the asterids, present since Turonian times, why do they dramatically diversify in the Tertiary and not earlier? Could the answer lie in the synergistic benefits of the co-occurrences of the apparent Tertiary origin of the herbaceous habit in certain asterids with associated short life cycle, the floral tube (extant since the Turonian in the asterid lineage), and modes of pollination associated with it (Fig. 7F)? Could speciation-amplifying synergy be related to yet other co-occurring temporally coincident characters? Could enhancements of the speciation process postulated to be associated with specific insect pollinators combined with vertebrate seed and fruit dispersers and their possible catalysis of allopatry also account for subsequent Tertiary radiations (Crepet, 1984; Tiffney, 1986)? We don't know. Yet these possibilities are consistent with the separate angiosperm radiations observed in the fossil record. And, in the context of character evolution and its timing in angiosperm history, is it unrealistic to assume that coordinated radiations between insect pollinators and angiosperms would occur only once, especially given that the Early Tertiary radiation included taxa with zygomorphic flowers (Fig. 7E), brush flowers (Fig. 7C, D), pronouncedly elongate corolla tubes (Fig. 7F), and presumed herbaceous habits?

Thus, a better understood, more conservatively interpreted fossil record now provides details of angiosperm history that preclude eliminating the importance of insect pollinators in angiosperm diversification and instead point to its potential significance. Yet, based on available evidence from neontological investigations of pollination ecology, one cannot assume that insect pollination was the single most important factor in angiosperm radiation or really be precise in assessing its relative importance among the array of possibly important angiosperm attributes. A better way to assess relative advantages of angiospermy in the context of paleontological data might be to carefully monitor the history of individual families and to assess the possible causes for speciation patterns within those families. This approach could ultimately give us more accurate insights into causes of angiosperm numerical species dominance and is within reach of the better fossil record of angiosperms that has come from stunning advances in fossil discovery combined with precise analytical techniques that now aid in accurately identifying fossil taxa.

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SELECTION OF FOSSILS FOR CALIBRATION OF MOLECULAR DATING MODELS[1]

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ABSTRACT

In the past decade, there has been a rise in interest in the plant fossil record. Fossils potentially provide information for assessing homology and evolutionary change (e.g., the popular missing link phenomenon), character evidence that affects phylogenetic conclusions and, thus, our understanding of modern relationships, evidence of past distributions that can aid in understanding biogeographic histories, and estimates of minimum ages of the clades to which they belong. Recently, many molecular biologists have used fossils in their analyses as a way of providing a calibration point for evolutionary models used to approximate ages for the nodes of phylogenetic trees. However, there has been little, if any, discussion of the criteria by which calibration fossils can be selected for these studies. When considering the use of a fossil as a calibration point, it is critical to take into account the quality of preservation, the method and details of identification (reliability of the taxonomic placement), and the accuracy of the published age. Here, we provide basic criteria for the use of fossils to calibrate molecular evolutionary models. These approaches not only provide better primary estimates for ages of clades, but also provide more reliable sources for those molecular biologists wishing to clean up their molecular clocks.

Key words: Age, calibration point, fossils, geology, identification, molecular clock, preservation, sampling.

Although fossil remains have been the objects of studies since Greek and Roman times, they are frequently ignored by researchers dedicated to molecular and development studies of plants and animals. Nevertheless, knowledge and interpretation of the fossil record are fundamental to understanding the history of life in general, including evolutionary processes. Fortunately, we are witnessing an explosion in the utilization of the fossil record, largely as a consequence of interest in ages of clades, as part of major biogeographical studies, and as a tool for assessing divergence times. Therefore, there has recently been a rise in interest in data obtained from fossils. Although the bulk of the fossil record remains undiscovered, there is no doubt that fossils potentially provide important information on diverse aspects of biology. Fossils are essential for understanding the sequence of evolutionary changes (e.g., the popular missing link phenomenon). They make available character evidence that affects phylogenetic conclusions and assessment of morphologic homology, and, consequently, they influence our understanding of modern relationships among taxa. They also provide support for past distributions that can aid in comprehending biogeographic histories. More importantly, they provide estimates of minimum ages of the clades to which they belong.

During the past 10 years, there has been a tendency to place fossils onto phylogenetic trees (with or without simultaneous cladistic analysis) to calibrate models estimating the age of the obtained nodes or clades (e.g., Bremer, 2000; Bremer et al., 2004; Donoghue et al., 2001; Renner & Meyer, 2001; Renner et al., 2001; Wikström et al., 2001; van Tuinen & Dyke, 2004; Yoo et al., 2005; and others). These methods, such as the nonparametric rate smoothing (NPRS; Sanderson, 1997), semiparametric rate smoothing by penalized likelihood (r8s program; Sanderson, 2002), and Bayesian divergence time estimation (Huelsenbeck & Ronquist, 2001), typically use fossils for calibration. Nevertheless, the fossils often are not included in the analyses performed using these models, and the placement of fossils requires either the results of previous morphological or combined cladistic analyses or acceptance of fossil identifications by paleobotanists. Fossils also have been used extensively for providing historical distributional data in biogeographical studies (Donoghue et al., 2001; Manos & Stanford, 2001; Morley & Dick, 2003; Renner et al., 2001; Wagstaff et al., 2002; Renner, 2004a, b, 2005; and others), but, once more, fossils are usually not included in the primary analyses and, instead, are simply mapped onto molecular phylogenetic trees based on assumptions about their relationships.

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The tendency to map fossils onto phylogenetic trees without including them directly in analyses makes the original identifications and quality of the fossils used extremely important. Although it is refreshing to see that fossils are utilized by molecular biologists to date their trees, it is also disappointing to see that sometimes they do not consider several issues when selecting fossils to be used as calibration points. Most will agree that merely mapping fossils on trees is not optimal because such placements are not directly tested and are, therefore, subjective. More importantly, any conclusion based on the position of phylogenetically untested fossils on a tree will be debatable. For example, Doyle and Donoghue (1993: 151) mapped the "most likely positions of Barremian-Aptian fossils" without directly including them in an analysis onto one of the most parsimonious trees obtained in a previous analysis of only extant taxa. Among the fossils were the earliest (early Cretaceous) putative monocots, represented by a *Liliacidites* Couper pollen grain and *Acaciaephyllum* Fontaine leaf. Later, Gandolfo et al. (2000) actually included the fossils within a cladistic analysis and found that *Acaciaephyllum* and *Liliacidites* were placed in various positions within various clades, but never in the paleoherbs clade in which Doyle and Donoghue placed them with the monocots. Thus, basing a most likely position for a fossil on a generalized identification without cladistic analysis, in this case, has not held up to later scrutiny.

In this paper, we will address various problems associated with fossil selection for calibration of molecular trees and some solutions that should minimize errors in selection of fossils. These recommendations are not meant to replace direct inclusion of fossils in a simultaneous total evidence analysis, which should be undertaken whenever possible, e.g., Hermsen et al. (2003) and Crepet et al. (2005).

The Problem

For any researcher of paleontology, it is common knowledge that working with fossils can be a tremendous challenge. This challenge has been made more complex by the need to either include fossils directly in modern cladistic analyses or provide strong evidence of their placement in the context of morphological synapomorphies for the groups in question. This often must be accomplished without the help of pre-existing morphological cladistic analyses, resulting in an increasing number of paleobotanical studies in which morphological matrices for modern groups must be constructed in order to place fossils (e.g., Nixon & Crepet, 1993; Crepet & Nixon, 1998a, b; Hermsen et al., 2003). Nixon (1996) reviewed several issues concerning the relationship between traditional paleobotanical work and modern cladistics, including issues of homology, missing data, and reconstruction. Clearly, some of these issues are aggravated when fossils are mapped, without inclusion in an analysis, onto previously calculated phylogenetic trees for calibration of molecular dating models. In a discussion focused on the surprising fossil divergence time between the ancestors of the birds and the mammals of 310 million years ago (Ma) proposed by Hedges et al. (1996) and based on molecular dating, Graur and Martin (2004) listed three sources of error that can influence clade age estimates: (1) errors in the topology of the phylogenetic tree, (2) errors in the taxonomic identification of the fossil material, and (3) errors in the chronological assignment of the geological strata. Combining these concerns about molecular dating per se with concerns about phylogenetic analysis of fossils (Nixon, 1996), we propose that there are several other factors that need to be addressed that impinge on each of the above caveats: (1) fossil preservation (the biased nature of fossil preservation, incompleteness of the fossils or fragmentary taxa, and reconstruction of fossil taxa); (2) methods of identification and taxonomic placement; (3) interpretation of the fossils and establishment of homologies; (4) sampling of taxa/fossils; and (5) the age assigned to the fossils based on the age of the formations where they were collected.

1. Fossil Preservation

Biased nature of fossil preservation. Plant fossils can be preserved as compressions, impressions, permineralizations, charcoalifications, and various other less common ways (Taylor & Taylor, 1993; Stewart & Rothwell, 2001). Each type of preservation provides different kinds of data and varying qualities of information. In a permineralization, the fossil usually has preserved internal structure (anatomy) providing details of cell types and tissues. However, some aspects of overall morphology may be difficult to interpret. In compressions, on the other hand, typically only characters of the external morphology and some cuticular features can be observed. Charcoalified (fusainized) fossils are an exception, since they are three-dimensional and mostly show a spectacular array of cell-by-cell preservation, providing a wealth of information unusual in the fossil record (Friis et al., 1986, 1992, 1994, 1995; Friis, 1990; Crepet et al., 1992; Herendeen et al., 1993, 1994, 1999; Nixon & Crepet, 1993; Crepet & Nixon, 1994, 1998a, b; Gandolfo et al., 1998a, b, 2000, 2002, 2004; Hermsen et al., 2003). It is common knowledge that soft parts are more difficult to preserve than hard

parts; there are more bones preserved than internal organs of vertebrates and more leaves and fruits than embryo sacs in flowering plants. Based on this, it is reasonable to assume that the majority of fossils are incomplete and, therefore, preservational bias is high. Preservational bias is also dependent on the environment in which fossilization occurs. Fossils are typically preserved better (with less damage and more detail of delicate structures) in environments of low energy (e.g., deltas and lagoons) than in areas of high energy (e.g., high-energy rivers and ash flows).

Incompleteness of the fossils or fragmentary taxa. Although plants typically have many parts that are relatively resistant to immediate decay and tend to preserve well, in general very few complete fossils with attached stems, roots, leaves, and reproductive organs are available in the fossil record. Notable exceptions occur with some aquatics such as *Archaefructus* Sun, Dilcher, Zheng & Zhou, presumably due to in situ preservation in low-energy environments (see Sun et al., 2002). This tendency to fossil fragmentation is further exacerbated by the tendency for some parts to be delicate and/or ephemeral on the living plants. Thus, we may have leaves, fruits, and wood from a single locality, but it is atypical to find a single fossil of the complete organism (attached fruits, leaves, and stems), much less the softer reproductive parts (i.e., flowers). Because of this fragmentary nature of fossils, many characters, even for well-known fossil taxa, are unavailable or incomplete. In the final analysis, most plant fossils are typically represented by single categories of organs (leaves, pollen grains, roots, fragments of stems, reproductive structures, etc.).

Isolated pollen grains, perhaps the most ubiquitous of plant fossils, provide a good example of the difficulties inherent in paleobotanical identification. Common characters studied in pollen grains are size, aperture number and type, and exine sculpture and structure, but determining taxonomic position based on these characters is often very difficult and in general has relied on direct comparisons with living taxa (Zavada, 1983, 1991) rather than comprehensive phylogenetic analyses. Isolated pollen remains difficult to include in large-scale phylogenetic analyses because the available characters that lend themselves to cladistic coding (e.g., aperture number and form) generally conform to very broad groups of taxa, and the details that differentiate smaller clades are often quantitative and labile, or relatively uniform (e.g., fagaceous pollen). Thus, without additional organs, assuming the occurrence of a taxon based on fossil pollen grains alone can be misleading (e.g., *Liliacidites*), such identifications must have less weight than identifications based on a broader range of features. The issue of identifications based on very few dispersed organs (e.g., pollen alone) is very important to address when choosing a fossil as calibration point, in view of the fact that typically only one organ is used for calibration and not the complete plant.

Reconstruction of fossil taxa. Another common practice among paleobotanists is to assemble dispersed parts, usually from the same or nearby localities, into full-organism or partial-organism reconstructions. A well-known case of reconstruction is the extinct genus *Glossopteris* Brongniart, based on an assemblage of variously preserved (but not directly attached) fossil organs including leaves, stems, roots, and a wide array of reproductive organs (e.g., Gould & Delevoryas, 1977). Another interesting case is the corystosperm group of Mesozoic seed ferns. Corystosperms have been considered to have *Rhexoxylon*-type wood based on the association of putatively corystospermous *Dicroidium* Gothan leaves with *Rhexoxylon* Bancroft emend. Archanglesky & Brett stems in the same strata and the presence of similar types of secretory cavities in the two fossils. However, *Dicrodium* leaves now have been found organically attached to fossil stems with *Dadoxylon* Enlitcher–type wood (Meyer-Berthaud et al., 1992, 1993), making the association with *Rhexoxylon* wood ambiguous. These two facts may imply that corystosperms have more than one type of wood; alternatively, perhaps *Rhexoxylon*-type wood is not actually found in the corystosperm clade. The reconstruction problem is even more aggravated for angiosperms. The most common angiosperm organs preserved in the fossil record are leaves and, less commonly, wood, seeds, fruits, and flowers. Unless all the organs are found in organic connection, it is very difficult to produce reconstructions of a whole plant, and there is a tendency to attempt reconstructions based on minimal data. Sometimes, fossils are preserved in the same sediments at the same horizon and they share one or more characters (e.g., the same type of stomata or venation pattern), and, in such cases, a reconstruction seems reasonable. This is the case with *Archaeanthus* Dilcher & Crane, which is based on five fossil taxa independently described by Dilcher and Crane (1984): *Archaeanthus linnenbergeri* (multifollicular fruits), *Kalymmanthus walkeri* (probably bud-scales), *Archaepetala beekeri* and *A. obscura* (putative perianth parts), and *Liriophyllum kansense* (leaves). These organ fossils were found associated but not in organic connection in narrow horizons in different localities, and, furthermore, all these fossils shared the presence of amber-colored resin bodies (Dilcher & Crane, 1984). Nevertheless,

without organic connection, reconstructions are always hypotheses to be tested with more data. One approach to testing hypothetical reconstructions is to include the disparate parts in a cladistic analysis to see if, indeed, they can be placed together in the context of parsimony based on the evidence present in each detached organ fossil. This approach was successfully used to test reconstructions of Cretaceous fern fossil organs by Gandolfo et al. (1997).

Another type of fossil reconstruction goes beyond connecting existing dispersed fossil organs and instead utilizes extrapolation of missing parts, usually based on the presence of attachment scars. Such reconstructions are the most highly suspect and, when the identification of the fossil in question relies on such extrapolations, should probably not be used as calibration points for molecular dating. One such extrapolated reconstruction is a putative Nymphaeales/Nymphaeaceae flower based on a single specimen that lacks petals/tepals and stamens. A reconstruction of these organs was presented, indicating filamentous stamens with basifixed anthers and elongate perianth parts based solely on the nature and position of attachment scars around the floral rim (Friis et al., 2001).

2. IDENTIFICATION AND TAXONOMIC PLACEMENT OF FOSSILS

Due to the uncertainty and ambiguity inherent in the fossil record, one of the most challenging aspects of paleontology is the identification and taxonomic placement of fossils. For example, during the late 1800s and until at least the 1980s, remains of leaves, flowers, and fruits of the Tertiary age were mostly described based solely on direct comparisons with extant genera and species based on the premise that for every given fossil there is one extant species that is most similar (Wolfe, 1973). These fossil species were often considered to be direct ancestors, or sister taxa, of extant species without explicit phylogenetic analysis and based on phenetic similarity alone. Unfortunately, these associations were too often based on vague characteristics, and the methodology, known as picture-matching, predominated in paleobotany until the 1980s. The use of this technique produced a great number of fossil misidentifications, as well as a high proportion of incorrect taxonomic placements (for further discussion, see Dilcher, 1973; Hermsen & Gandolfo, 2004).

The most problematic aspect of poor reconstructions, fossil misidentifications, and taxonomic misplacements results from the use of suspect fossil data by non-paleobotanists in studies that utilize fossil occurrences to estimate ages of clades. Such uses are not restricted to molecular dating but also include biogeographical studies in which the age of clades may be used to postulate historical patterns of vicariance and dispersal. Unfortunately, the fossils used in such studies are often not critically evaluated and the identifications and taxonomic affinities are taken for granted from the literature. An example of this problem is provided by Schultheis and Donoghue (2004), who used a fossil assigned to the modern genus *Ribes* L. (*Ribes* species *Axelrod* 1996, UCMP 11156; Fig. 1A) as the oldest reliable fossil occurrence of *Ribes* in North America in a study of the molecular phylogeny and biogeography of *Ribes*. However, a subsequent careful study of the fossil in a review of the group by a paleobotanist revealed a lack of consistent characters with modern *Ribes*, and this calls into question (Hermsen, 2005) any conclusions based on the previous misidentification (Fig. 1A). Another example is a fossil identified as the putatively araceous monocot *Arisaema herperia* Knowlton 1926 (USNM 36885; Fig. 1B). Subsequently, *A. herperia* was used to estimate the age of the Tertiary floristic link between Asia, North America, and East Africa based on a Bayesian chloroplast phylogeny (Renner et al., 2004). The *A. herperia* fossil is based on a single specimen, and Knowlton's identification was performed using the picture-matching method of gross similarity. Hermsen and Gandolfo (2004) reexamined *A. herperia* and found a lack of sufficient characters to make a definitive taxonomic placement with *Arisaema* or the family Araceae. Furthermore, in a previous paper not cited by Renner et al., *A. herperia* had been removed from *Arisaema* and reassigned to *Liquidambar* L. by Brown (1946). Although the taxonomic position of this fossil is still in dispute (Renner et al., 2004; Hermsen & Gandolfo, 2004), it illustrates the difficulties that paleobotanists face in identifying and assigning fossils and the problem of uncritical acceptance of fossil identifications from the older literature. Pollen grains are also frequently used as calibration points (e.g., Bremer, 2000), and these present a challenge as well (see paragraph above).

Different interpretation of the fossils and the establishment of homologies. Nixon (1996) discussed in detail issues of homology assessment in fossils; these will not be repeated here. Different researchers often interpret the same fossil structures differently, sometimes dramatically so, and this increases the uncertainty of homology. Imperfect preservation, lack of context of detached parts, missing features, and lack of precise detail (e.g., anatomy) all contribute to more complex and difficult homology assessment in fossils. Nixon (1996) provided several examples of these issues, including differing interpretations of the homology of the multiovulate cupules of Mesozoic seed ferns such as

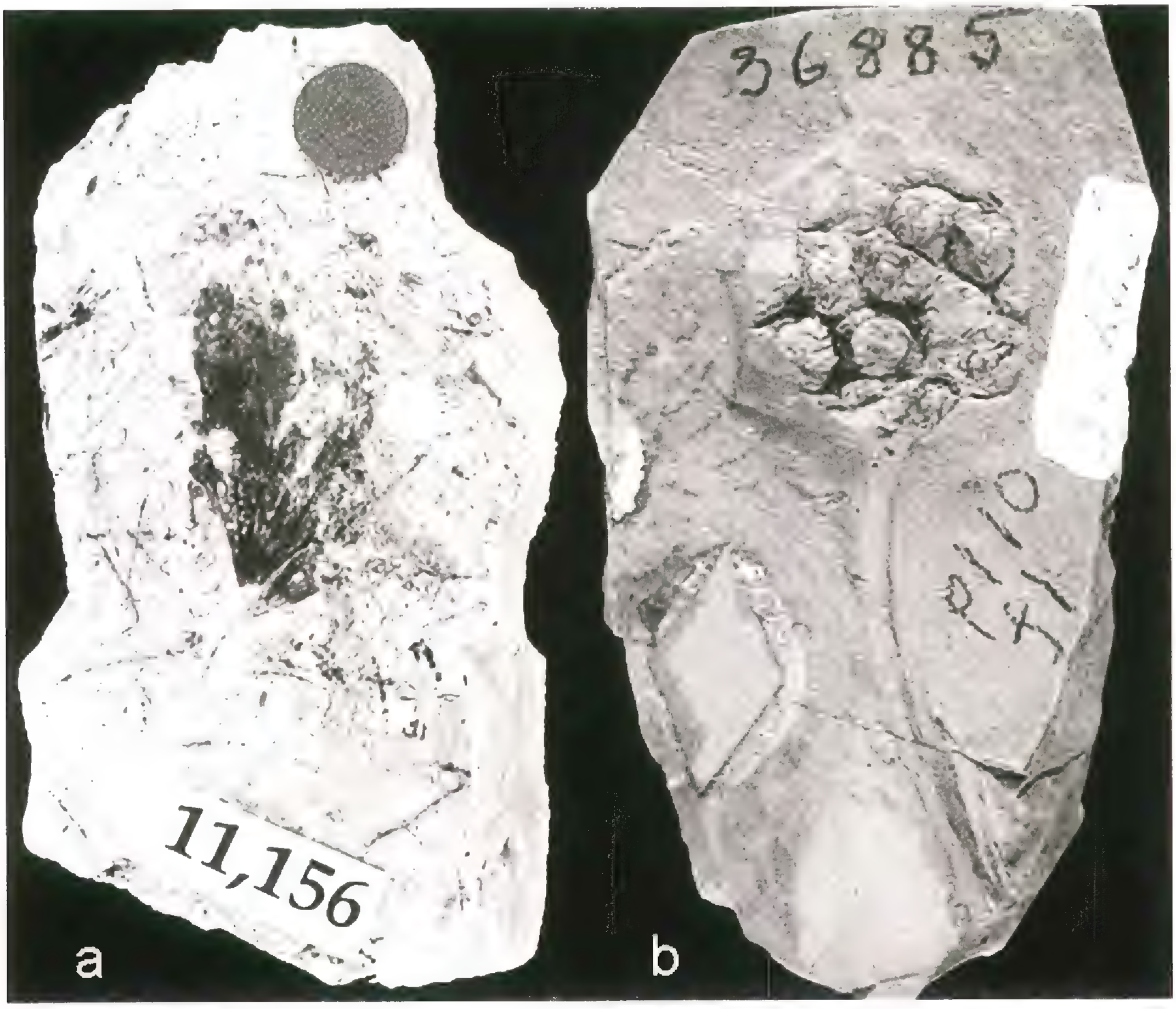

Figure 1. —a. *Ribes* sp. (UCMP 11156) housed at the University of California Museum of Paleontology, Berkeley, California. —b. Type specimen of *Arisaema heperia* Knowlton (USNM 36885) housed at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Caytonia Thomas as compared with angiosperm ovules. Undoubtedly, differences in interpretation may be influenced either by different views of background knowledge (e.g., a given scenario of morphological change) or may be a form of verificationism—fitting our assessment of homology to reflect a given (often popular) view of phylogenetic history. Even when fossils are considered to be very well preserved, there are a limited number of characters one can investigate, and homology assessments are in general weaker than with the extant plants used for comparisons (Nixon, 1996).

3. FOSSIL SAMPLING

Another aspect that has added confusion to the process of the selection of fossils for calibration is the spotty and incomplete fossil record or sample. The incompleteness of the record is apparent in at least three dimensions: temporal, geographic, and taxonomic. Originally, paleontological collections were secondary to particular research programs, and most paleobotany collections were maintained by scientists coming from different backgrounds. The majority were geologists gathering data for physical geological purposes or for commercial endeavors such as mines and oil prospecting. Therefore, the information recorded for specimens within these collections (exact locality, strata and type of environment in which deposition occurred, other fossil associations, etc.) was minimal. As with older herbarium labels, old fossil collections often lack precise location data and other informative facts, and sometimes the information provided on the labels was incorrect. Today, fossil collections are usually made with an emphasis on careful stratigraphic mapping and, more recently, global positioning system waypoints (GPS data) to allow fairly exact relocation of localities and quarries.

Special care is put into documenting vertical and horizontal variations, and, usually, extensive and intensive sampling is undertaken at productive sites. This approach increases the probability of collecting more than a single specimen per taxon, and therefore, taxonomic assignment and identification are more accurate. This more thorough sampling also reduces the proportion of missing data when multiple documented specimens from the same site become available.

4. GEOLOGY AND AGE

Perhaps one of the most pervasive issues related to the utilization of fossils for calibration of molecular dating methods is the tendency to accept the original description, taxonomic assignment, and geologic dating without further investigation (Hermsen & Gandolfo, 2004). As with plant classifications, classification of geological groups, formations, strata, horizons, etc., and their assigned (calculated) age are not static. Thus, when considering a fossil for calibration of a molecular tree, it is necessary to thoroughly search for later modifications of both the taxonomic assignment and, just as importantly, changes in the assigned age. The early angiosperm *Archaefructus* is a good example of an original geologic age that was disputed and subsequently changed from Late Jurassic to Early Cretaceous based on additional data (Sun et al., 2002).

Other issues are purely terminological. Many competing stratigraphic schemes were used until around 1880, when geologists started to address the problem of the necessity of a standard stratigraphic scale. Although it took them more than 100 years to produce the guidelines for defining global chronostratigraphic units, the International Commission on Stratigraphy ([ICS], a commission within the International Union of Geological Sciences [IUGS]) produced the first Global Stratigraphic Chart (Cowie & Basset, 1989), which is revised frequently. Different geologic dating methods often produce different estimated ages for the same deposits. It is now generally accepted that the most accurate ages are obtained by paleomagnetic and radiometric data (U-Th-Pb, Rb-Sr, K-Ar). However, it is perhaps more common to date by other means, such as stratigraphy, biostratigraphy, palynology, and presence or absence of indicator taxa or assemblages of taxa in combination. Both stratigraphic and biostratigraphic (floristic) dating have caveats, including unconformity of strata and the potential for what are considered to be unique floral assemblages to persist or reappear. Therefore, depending on what method has been used to calculate the age of strata, the assigned age and method should be carefully considered when selecting calibration fossils. A clear example of this is the Laguna del Hunco paleoflora that occurs in Patagonia, Argentina. Initially, its age was considered Miocene (23.03–5.33 Ma) based on comparisons with other paleofloras of South America (Berry, 1932), but, based on argon/potassium dating, Archangelsky (1974) suggested a Paleocene age (65.5–55.8 Ma). Mazzoni et al. (1991) later estimated the age at 51–43 Ma based on ignimbrite radiometric dating, and, most recently, Wilf et al. (2003), using paleomagnetic data, determined the age to be early Eocene (52.8–51.7 Ma). Depending on which of these age ranges are used, fossils from this site might produce very disparate results if used for calibration of molecular trees.

The problems of the most accurate date for a particular locality or stratum not only are dependent on dating methods but also are related to the instability of the geological scale, which has changed through time. In 1937, most researchers agreed that the Tertiary started 70 Ma, but today we accept 65.5 Ma as the basal age for this period. Overall, the assignment of the age of the beginning of the Tertiary has fluctuated between 70 and 63 Ma. In addition, the duration of the periods within the Tertiary has changed, with the Oligocene having a 16-million-year duration in 1937 (from 48–32 Ma) to a 17.4-million-year duration in 1982 (from 38–24.6 Ma), and today it has 10.87-million-year duration from 33.9–23.03 Ma (Gradstein et al., 2004; Table 1). Such differences, if not considered when using fossils as calibration points, can also result in widely differing estimates of ages. This kind of variability is observed for all the eras, periods, and stages of the geological scale (Gradstein et al., 2004).

The Solutions

It is impossible to completely eliminate problems associated with selection of fossils as calibration points for molecular dating, but such problems as discussed above can be reduced to a minimum if certain steps are taken. This should reduce the errors introduced into such analyses and provide more repeatable results. Undoubtedly, some or perhaps most of the suggested steps that require a paleobotanist or paleobotanical or taxonomic knowledge to undertake may be beyond the scope of many studies. We would hope that the same care and concern should go into the selection of fossils as that which goes into the selection of appropriate genes, methods of phylogenetic analysis, and taxon sampling in the original studies. In many ways, the final result of molecular dating may be more dependent on the selection of a calibration fossil than on the particular

Table 1. Comparison at the period level of selected Paleogene time scales from 1937–2004; modified from Gradstein et al. (2004). Age in million years (Ma). B, beginning of period; E, end of period; D, duration of period.

	Paleogene								
	Paleocene (Ma)			Eocene (Ma)			Oligocene (Ma)		
	B	E	D	B	E	D	B	E	D
1937	70	68	2	68	48	20	48	32	16
1960	70	60	10	60	40	20	40	25	15
1972	65	53.5	11.5	53.5	37.5	16	37.5	22.5	15
1978	65	53.5	11.5	53.5	37	16.5	37	24	13
1982	65	54.9	10.1	54.9	38	16.9	38	24.6	13.4
1985	66.5	57.7	8.8	57.7	36.5	21.2	36.5	23.6	12.9
1987	66.5	54	12.5	54	36	18	36	25.2	10.8
1990	65	56.5	8.5	56.5	35.4	21.1	35.4	23.3	12.1
1995	65	54.5	10.5	54.5	33.7	20.8	33.7	23.8	9.9
2004	65.5	55.8	9.7	55.8	33.9	21.9	33.9	23.3	10.6

gene sequences utilized for the phylogeny, assuming that multiple sequences are selected.

RECOMMENDATION 1: TAXON SELECTION

Verification of the fossil identification:

(a) If possible, verify the identity of the fossil with qualified paleobotanists. Often, suspect fossil taxa may be well known to the paleobotanical community, but there may be relatively little published on these subsequent to the original description. For those who are qualified, it is, of course, better to directly study the actual specimen(s) if possible.

(b) Evaluate the original diagnosis provided by the author of the fossil genus/species. In many older papers, fossils lack a formal diagnosis, and such fossils are immediately suspect in terms of their identity. At a minimum, some characteristics that are unique to the group to which the fossil is assigned should be determined. If it is not possible to characterize the fossil with a diagnosis that clearly associates it with its presumed modern relatives and eliminates assigning it to multiple other taxa, it should probably be excluded immediately as a candidate for a calibration point.

(c) Check for all taxonomic/nomenclatural changes subsequent to the original publication. Sometimes, this is sufficient to eliminate a fossil from consideration.

(d) Check for synapomorphies that provide unequivocal taxonomic assignment of the fossil. If the fossil lacks synapomorphies or at least an assemblage of characters that confirmed its taxonomic placement, it should be rejected and not used as a calibration point.

(e) If the fossil taxon has not been previously included in a phylogenetic analysis, it should be, if at all possible. This can often be done by including the fossil taxon in an existing morphological analysis of modern taxa that are within the scope of the identification.

RECOMMENDATION 2: FOSSIL SOURCE

(a) Confirm the data associated with the collection of the fossils: formation, strata, and locality. Initially, these should be obtained from the original paper, but because concepts of formation and strata can change, these need to be verified.

(b) Verify that the specimen(s) are still available. This may require contacting the presumed collection in which the specimen is housed. Any fossil taxon used as a calibration point should have available specimens and, presumably, a clearly designated type specimen, so that future studies can evaluate the quality of the assignment.

RECOMMENDATION 3: AGE

(a) Confirm the age of the associated formation, strata, and locality. Again, because concepts change and strata are sometimes remapped, it is important to determine if any changes have occurred since publication of the fossil.

(b) Check in the geological literature for changes in the estimated age of the strata since the date of original publication of the fossil.

(c) Use the youngest, most probable, most precise period (if the period is Eocene, use 33.9 Ma and not 55.8 Ma).

(d) Provide the correct bibliographic citation for the age adopted and, in particular, the source of any revised age estimates for the strata in which the fossil(s) occur.

Conclusions

The use of fossils as calibration points in molecular dating is an exciting and potentially useful endeavor, independent of which molecular dating methods are used or the theoretical justifications for particular approaches. The renewed focus on fossils in this context has exposed numerous issues that have been well known to paleobotanists, but may not be apparent to workers seeking to simply obtain a calibration point as easily as possible. Providing carefully identified and accurately aged fossils that can be utilized by molecular biologists in such studies is the responsibility of the paleobotanist. Unfortunately, the vast body of paleobotanical work is largely inappropriate for use to calibrate molecular trees without careful scrutiny and attention to issues related to traditional, often nonrepeatable, approaches to fossil study. The paleobotanist must be a primary partner in these endeavors, and, hopefully, paleobotanists will increasingly become collaborators in studies that use molecular dating methods. At a minimum, molecular botanists should carefully consider which fossils they are using, and paleobotanists should seek to provide fossils in a verifiable phylogenetic context based on combined molecular and morphological analyses. In these ways, both fields of endeavor will be improved.

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PALEOBOTANY, EVIDENCE, AND MOLECULAR DATING: AN EXAMPLE FROM THE NYMPHAEALES

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ABSTRACT

In recent years, most systematics studies have focused on phylogenetic analyses of molecular data sets. The latest trend has been to add molecular dating to these phylogenies utilizing methods such as nonparametric rate smoothing (NPRS) and penalized likelihood (PL) and calibrating these analyses using (often only one or very few) fossils. The success of such approaches is dependent on several assumptions, including a local clocklike behavior of evolution, the accuracy of the phylogeny, the correct phylogenetic placement of fossils, and the consistency of particular fossils in extrapolating rates throughout a given phylogenetic tree. An example of such an analysis of the Nymphaeales is provided to illustrate inappropriate use of fossils in this context and faulty results based on inadequate and/or inappropriate analyses. Neither fossil identifications nor a particular method of molecular dating should be called into question based on the disparity of a single analysis. Indeed, fossil observations and molecular dating are often at odds due to failure of the data to meet minimum assumptions of a clocklike behavior and poor or inadequate sampling of extant taxa, molecular sequence data, and/or fossils. Rejection or acceptance of either the fossils or the molecular dates resulting from their use should be considered in light of direct analysis of the fossils and compared to other analyses using other fossils and/or other extant data sets. Rejection of fossils based on unexpected results is merely verificationism.

Key words: Angiosperm, molecular clock, nonparametric rate smoothing, Nymphaeales, penalized likelihood.

In recent years, the fields of plant and animal systematics have progressed from a relatively subjective and often authoritarian process of classification (see Cronquist, 1981) to one based on monophyletic groupings following the recommendations of Hennig (1966), although proponents of paraphyletic classifications persist (e.g., Brummit, 2006; Hörandl, 2007). The existence of comparative DNA sequence data for most major taxa now provides a basis for coordinating phylogenies of extant species and fossils via simultaneous molecular-morphological analyses that include both living and extinct terminal taxa (numerous papers, including Sun et al., 2002). In turn, such combined analyses (or surrogates that attempt to place fossils on independently calculated phylogenies) can be used to estimate minimum ages of hypothetical ancestral nodes (Crepet et al., 2004). A recent and increasingly popular use of this approach is to use fossil calibration points to estimate evolutionary rate parameters for trees on the basis of molecular data and thus extend the dating of nodes beyond the immediate attachment point of the fossils (Sanderson et al., 2004). These methods can also be used to calculate statistical confidence intervals for these estimated ages. In the broadest sense, such analyses rely on what was originally referred to as the molecular clock—the assumption that evolutionary rates of particular genes are constant either globally (the ideal situation) or sufficiently within parts of the tree to be amenable to models that attempt to minimize rate change from one node to another. This idea, that evolution may occur at least locally in a clocklike manner, can be traced back at least four decades (Zuckerkandl & Pauling, 1962, 1965), although the recent discussion of this topic in the context of phylogenetic analysis has shifted somewhat, and several issues deemed important in the original dialogue (such as gene neutrality; see King & Jukes, 1969) are seldom mentioned in current discussions. In many ways, the idea of a molecular clock underlies all currently used model-based approaches such as maximum likelihood or Bayesian phylogenetic analysis, which rely on predictable patterns of molecular change that can be designated in a model with relatively few parameters. Clocklike evolution is also the underlying justification for using neighbor-joining trees as an approximation of phylogenetic pattern, based on a model of minimum evolution and a least-squares–based algorithm (Saitou & Nei, 1987).

It is not the intent of this paper to review or criticize various methods of node-dating using molecular trees calibrated with fossil data; indeed, some of the proponents of these methods already have provided relatively strong criticisms and caveats (Sanderson et

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al., 2004). To a great extent, the acceptance or rejection of molecular-dating approaches is based on faith in the general concept of the molecular clock and the accuracy and/or reliability of particular models that are incorporated into such methods. In many cases, age estimates of important nodes based on molecular dating are far older (and only rarely, younger) than minimum age estimates based on existing fossil evidence alone (see an excellent review of these issues by Graur & Martin, 2004). Of interest here is not whether molecular dating is accurate but, instead, what the relationship between these two approaches is and how to resolve discrepancies in results. The two approaches can be summarized as follows: (1) Estimation of the minimum ages of clades is based on direct observations of fossils—their identification in a phylogenetic context—combined with dating of the strata in which they occur and subsequent estimation of minimum ages for clades based on the oldest fossil record included by that clade (e.g., Crepet et al., 2004). This method may or may not involve additional extrapolation of ages based on branch lengths. The phylogeny on which the estimations are based might be molecular, morphological, or combined, but there must be some way to explicitly assign fossil taxa to positions in the tree. (2) Estimation of ages is based on molecular-generated branch lengths and topologies interpreted with a particular algorithm (and implicit model), such as penalized likelihood (PL) or nonparametric rate smoothing (NPRS; Sanderson, 1997), and calibrated with one or more fossils identified and dated by the above methods. A molecular tree must be available, and, in general, there has been little discussion of the exact requirements for fossil selection and how to determine exactly where it should be placed in the tree (see Gandolfo et al., 2008).

In order to discuss problems that arise when these two approaches yield different answers, I will use a particular example that involves fossils that I have studied directly to illustrate some of these issues.

Background: The Molecular Clock

It is generally accepted that the idea of the molecular clock was first introduced by Zuckerkandl and Pauling (1962, 1965). Soon after, in the mid- to late 1960s, the idea of molecular clocks and the potential to use such clocks for constructing phylogenetic trees, estimating evolutionary rates, and/or estimating the age of particular clades emerged. Because DNA sequencing was not feasible at that time, most of the discussion of molecular clock tree construction and dating centered on (very scarce) protein sequence data, particularly cytochrome C. One major assumption of the original approach to molecular clocks was that neutral genes or sites, and what can be termed synonymous changes at these sites, were the most likely to have clocklike rates and thus were the best candidates for phylogenetic reconstruction. It is important to note that the concept of (selective) neutrality in this context excludes both sites that are variable (and selected for or against in various ways), as well as, and perhaps most importantly, sites that are conserved (e.g., stabilizing selection) and show little variation. A truly neutral site is one in which selection is absent, whether it varies much or little.

At one point, the idea that most or all evolutionary changes are actually (or virtually) neutral also entered the debate. This neutral theory—the idea that most evolutionary change is non-Darwinian—is closely tied to the molecular clock, as can be seen in the following quotation (King & Jukes, 1969: 796): "The rate of non-Darwinian change equals the rate of selectively neutral mutation and is independent of environmental fluctuations and of population size. For a given protein, the rate of such change should be nearly constant. Darwinian change, in contrast, is under the influence of changing environment, adaptive radiation, fluctuations in population size, and such factors as adjustment to major changes in the genetic background. Thus it might well be subject to bursts of rapid change in some species and relative stability in others."

Following this reasoning, genes (or individual nucleotide bases) undergoing selective (Darwinian) evolution will be poor candidates for a molecular clock. Sites under selective pressure will have heterogeneous rates, both vertically within clades and horizontally among clades. Such sites will not exhibit clocklike behavior in their rate of evolutionary change.

Some recent papers have suggested that bases at codon third positions have more of a clocklike pattern of evolutionary rate than first- or second-position bases (e.g., Mercer & Roth, 2003). This is intuitive—if substitutions in third positions are synonymous in terms of the proteins coded for, then they would be neutral in the sense of the above discussion and might be better candidates for modeling a molecular clock. The potential for third-position bases to provide phylogenetically informative variation has also been debated, and Källersjö et al. (1999) found that more groups were supported in one large molecular analysis by (presumably neutral) third-position bases than by first and second positions. This may be due to rate issues (because they are neutral and evolve faster, variation in third-position bases resolves more terminal groups). At any rate, the issues of saturation and the Felsenstein zone (see Felsenstein, 1978) have

generally been invoked to suggest that third positions are less useful due to repeated substitutions. These issues are not meant to be addressed here but certainly are necessary to an understanding of how molecular dating might perform with different data sets and different analyses (e.g., parsimony vs. maximum likelihood).

In a recent review of the subject, Benton and Ayala (2003) did not address the primary issue of whether evolutionary rates are clocklike or issues related to Darwinian versus non-Darwinian evolution. Instead, they framed the problem as a conflict between results from fossils providing dates that are younger and molecular clock methods providing dates that are much older. Anecdotally, they provide an example where, with additional data and analyses, the two disparate estimates come closer together, and they present this as a hopeful indication that we are on the road to reconciling the problems associated with molecular clock dating.

It is important to note that although some of the most popular molecular-dating methods, e.g., NPRS (Sanderson, 1997), on the surface may not seem to require rate constancy, they do assume local rate constancy as opposed to global rate constancy. If rates are completely unpredictable (i.e., poorly correlated with time) and/or change rapidly and frequently within and among clades, even methods such as NPRS will fail to provide results. Other issues related to the selection of suitable fossils to calibrate molecular dating are discussed elsewhere in this issue (Gandolfo et al., 2008).

An Example: The Nymphaeales

In the past two decades, molecular analyses of angiosperms have placed various modern groups as concordant sister taxa to the bulk of the extant angiosperms, ranging from *Ceratophyllum* L. based on *rbcL* alone (Chase et al., 1993; Nixon, 1999) to various permutations with *Amborella* Baill. as the sole sister taxon of all remaining extant angiosperms (e.g., Soltis et al., 2000). In these latter trees, the nymphaealean clade, along with Illiciales and Austrobaileyales, is generally placed as a sister group of all remaining angiosperms excluding *Amborella*. It should be noted that statements regarding these trees often suggest that *Amborella* (or *Ceratophyllum*) is a basal or primitive angiosperm, which of course is merely a misinterpretation of sister-group relationships (such terminology in the context of fossils has more meaning; Sun et al., 2002). It is easily understood that if the three-gene tree (Soltis et al., 2000) is correct, then *Amborella* and any extant angiosperm diverged from the common ancestor of angiosperms at the same time, and therefore the lineage culminating in *Amborella*, have had the same amount of time to diverge from the ancestral angiosperm as any orchid, mistletoe, grass, or Texas bluebell. *Amborella* is no more basal within the angiosperms than any other extant species of angiosperm. Because of their aquatic nature and presumably early divergence (whether termed basal or not), the Nymphaeales has attracted considerable attention in the context of the putatively primitive angiosperms. Indeed, this interest has increased with the discovery that some of the earliest identifiable angiosperm fossils appear to be aquatic (Sun et al., 2002).

A recent paper by Yoo et al. (2005) that focuses on Nymphaeales provides a useful example of the conflict between traditional paleobotanical evidence and the results of molecular-dating methods. Yoo et al. (2005) used four approaches (strict molecular clock, NPRS, PL, Bayesian) in an attempt to calculate divergence times for the modern crown group of Nymphaeales as well as the age of the angiosperms. They utilized a previously published morphological cladogram of Nymphaeales (Yoo et al., 2005: fig. 1, from Les et al., 1999). This tree was used to map molecular changes using sequences for *rbcL*, *matK*, and 18S that were downloaded from GenBank and aligned using CLUSTAL X (Thompson et al., 1997) with the default options, and the alignment was then adjusted manually (Yoo et al., 2005). This tree has 16 terminals, including four gymnosperm outgroups (*Ginkgo* L., *Gnetum* L., *Larix* Mill., *Taxus* L.), *Amborella*, the Illiciales or Austrobaileyales represented by three terminals (*Illicium* L., *Schisandra* Michx., *Austrobaileya* C. T. White), and the eight commonly recognized genera of Nymphaeales (*Victoria* Lindl., *Nymphaea* L., *Euryale* Salisb., *Ondinea* Hartog, *Barclaya* Wall., and *Nuphar* Sm. from the Nymphaeaceae, and *Cabomba* Aubl. and *Brasenia* Schreb. from the Cabombaceae).

It is important to note that Yoo et al. (2005) did not calculate a new cladogram based on the data matrix that they generated from downloaded sequences, but, instead, the pre-aligned molecular sequences were superimposed on the morphological tree from Les et al. (1999). The program r8s (Sanderson, 2003) was then used to analyze the molecular matrix using the Les et al. (1999) tree as the input tree. It is not clear why Yoo et al. (2005) did not attempt a new analysis of the molecular data, given that the original data matrices needed to be included in order to estimate molecular divergence times. They then analyzed the matrix/tree combination with various methods of molecular dating available in r8s: "[B]ecause all tests of rate heterogeneity among lineages were highly significant, we used three approaches that have been

proposed for use with heterogeneous rates, NPRS (Sanderson, 1997, 1998), PL (Sanderson, 2002), and a Bayesian method (Thorne et al., 1998; Kishino et al., 2001; Thorne & Kishino, 2002)" (Yoo et al., 2005: 695).

The results of the molecular dating analysis by Yoo et al. (2005: 697) suggested a relatively recent Tertiary divergence for the crown group of Nymphaeales, even though Cretaceous fossils had been previously described for the family Nymphaeaceae: "Our divergence time estimates indicate that extant Nymphaeales diversified relatively recently, whereas the stem lineage to Nymphaeales is old, based on a fossil attributed to Nymphaeales from the Early Cretaceous (125–115 mya; Friis et al., 2001) and a fossil attributed to Nymphaeaceae from the middle Cretaceous (~90 mya; Gandolfo et al., 2004)." In other words, the age of diversification found by Yoo et al. (2005) for Nymphaeales based on molecular-dating methods is considerably younger than the published fossil evidence (in contrast to the more common situation, where fossils are typically younger than estimated ages based on molecular trees). Oddly, Yoo et al. (2005: 697) use the rare but similar contradictory results found in other studies to validate their own results: "These results for Nymphaeales indicating recent diversification in an ancient lineage agree with similar findings for the basal angiosperms Chloranthaceae (Zhang & Renner, 2003) and *Illicium* (Illiciaceae; A. Morris, unpublished data). The fossil record indicates clearly that Chloranthaceae represent one of the oldest angiosperm lineages, with unequivocal reproductive structures resembling those of *Hedyosmum* from the Barremian Aptian boundary, approximately 125 mya (see Friis et al., 1994, 1999; Friis, 1997; Doyle et al., 2003; Eklund et al., 2004, for recent interpretations and lists of earlier references). However, divergence time estimates based on molecular data indicate that the extant genera of Chloranthaceae are relatively young (i.e., 60–29 mya for *Hedyosmum*, 22–11 mya for *Chloranthus*, and 18–9 mya for *Ascarina*; Zhang & Renner, 2003)."

There seems little point in belaboring the faulty logic in citing agreement with other, unrelated analyses in which fossil ages are older than the age of crown groups estimated by molecular models. Usually, when molecular dating methods repeatedly provide significantly older dates than fossil evidence, the argument of an incomplete fossil record is typically voiced to explain the discrepancy. In contrast, since in their study Yoo et al. (2005) found significantly younger diversification of the crown group for Nymphaeales than well-studied fossils, they were forced to decide that the fossil identifications were incorrect. Unfortunately, for this interpretation, in the case of the Laurales example, the Chloranthaceae have a long unequivocal fossil record with some fossils that have the same characters as modern *Hedyosmum* Sw. from the early Cretaceous (Eklund et al., 2004). However, molecular clock dating tells us this cannot be so; according to the rate model(s), *Hedyosmum* did not diversify until much later. The pattern here is apparent—if the dates are too old then we just do not have enough fossil data; if the dates are too young, then the fossils are misidentified. In other words, no independent tests of the molecular dates are allowed, and fossils are the only independent tests. Apparently, the particular results of the molecular dating are not called into question in cases of conflict, only the fossil evidence.

THE AGE OF ANGIOSPERMS

Apparently in order to provide evidence that both nymphaealean fossils from the Cretaceous (Friis et al., 2001; Gandolfo et al., 2004) were erroneously placed, Yoo et al. (2005: 695) then compared calibration of the age of the entire angiosperm clade using these two fossils (in two independent PL analyses) in r8s. Given the previous putative overestimation of the age of Nymphaeales, the results were rather shocking: the angiosperm clade diverged 1093 million years ago (Ma; more than one billion years ago) based on *Microvictoria* Gandolfo, Nixon & Crepet (Gandolfo et al., 2004) and 1457.3 Ma (almost 1.5 billion years ago; Yoo et al., 2005: table 2) based on the Friis et al. (2001) fossil. Again, Yoo et al. (2005) suggested that this provides evidence that the fossils are phylogenetically misplaced. As an aside, note the high precision for the age of angiosperms based on the Friis et al. (2001) fossil calibration (to ca. 300,000 years of almost 1.5 billion years).

Yoo et al. (2005: 700) proceed to use the poor performance of PL (in the sense of providing what might be considered wildly inaccurate estimates of the origin of angiosperms) as an indictment of the placement of *Microvictoria* as a crown group Nymphaeaceae fossil: "There seem to be two possible explanations for the disparity between the fossil record for angiosperms and the age inferred here using PL and *Microvictoria*, as placed within Nymphaeaceae by Gandolfo et al. (2004) as the calibration point. Either methods of estimating divergence times from molecular data are highly dubious or the phylogenetic placement of *Microvictoria* in Gandolfo et al. (2004) may need to be reconsidered."

There are more than two possible explanations for the problematically old estimates calculated using PL for the origin of angiosperms, and the dichotomy of two possible explanations presented by Yoo et al. (2005) is a false one. First, the fact that in a particular

instance a method fails does not make it highly dubious, and certainly this is not a reasonable conclusion. This is simply a classic case of a straw man, clearly intended to steer the reader toward the second alternative explanation provided. Maximum likelihood, parsimony, and almost any conceivable phylogenetic method fail under certain circumstances (e.g., long-branch attraction or repulsion, a.k.a. the Felsenstein zone or the Farris zone; Siddall, 1998), and this fact alone does not impugn either of these widely used methods. Indeed, with any method one expects failure a certain percentage of the time, particularly when the assumptions of the method are not met. Certainly, there is a strong possibility that evolution is not acting in a sufficiently clocklike manner in the region of Nymphaeales relative to other parts of the angiosperm tree, and/or the rates in this part of the tree are not representative of rates elsewhere. Such heterogeneity of rate could result in fossils from these clades being poor choices for calibration of an entire seed plant cladogram, and this is actually the best alternative explanation. In other words, it may very well be that rates can differ dramatically between ancestor and descendant nodes, and using a single fossil from an area of the tree that is heterogeneous for rates and not typical of average rates elsewhere will likely result in estimates that are obviously wrong. Given that Yoo et al. (2005) had already established the high significance of rate heterogeneity among lineages, it seems odd that they did not provide this obvious possible interpretation of their results.

In addition to the third explanation above (assumptions of PL were not actually met due to highly heterogeneous rates), there is also the possibility, for those who adhere to the primacy of molecular-dating methods, that the angiosperms are much older than previously thought (interestingly, in the majority of results of molecular dating, this is the finding—that the age of major groups is much older than previously thought—yet, in those cases, the argument is typically provided that we merely lack fossils of sufficient age; see Graur & Martin, 2004). It seems that when ages are acceptably older than expected based on fossil evidence alone, molecular dating is working well, and, when they are unacceptably older than expected, the fossil record (or identification) is bad.

If one accepts the general validity of molecular-dating methods, as well as the identification of the nymphaealean fossils by two different paleobotanical research groups (Friis et al., 2001; Gandolfo et al., 2004)—one of which was included in cladistic analysis—the "too old" (Yoo et al., 2005: 699) angiosperm results might suggest two interpretations. First, perhaps there were significant changes in rate in the Nymphaeales lineage, e.g., a rapid diversification in the Cretaceous, followed by much slower rates within the crown groups. Second, a single fossil from a particular clade might not be sufficient to provide accurate results using molecular-dating methods. These interpretations (not considered by Yoo et al., 2005) do not impugn molecular dating per se or traditional paleobotanical observation (which, after all, must be the basis for calibration of trees used in molecular dating). The idea of highly heterogeneous rates, with rapid diversification followed by relative stasis, was popularized a few decades ago by Eldredge and Gould (1972) when they championed the concept of punctuated equilibrium. Perhaps this has fallen out of favor because it is difficult to model and, if demonstrated to be common, would cause severe problems with simple rate-smoothing methods, which would tend to over-equalize rates among ancestors, descendants, and disparate clades. In this context, based on current large-scale phylogenetic trees, it is likely that the original angiosperms were not fully aquatic (Crepet et al., 2004), and it is possible that there was a period of rapid diversification in the nymphaealean clade in invasion of the aquatic habitat, followed by subsequent relative stasis in a stable aquatic habitat.

Yoo et al. (2005: 700) provide a rather complicated explanation of why *Microvictoria* might have been misplaced: "Alternatively, *Microvictoria* may be misplaced in the phylogenetic analysis of Gandolfo et al. (2004), perhaps due to homoplasy in the crucial morphological characters scored and included in that study. That is, a now-extinct assemblage of early angiosperms may have possessed suites of traits not found in any extant groups." This speculation needs little discussion. There might be any number of clades that are extinct and never discovered as fossils that any particular existing fossil might be related to. The same can be said for any extant taxon as well. There is always the possibility of something as yet unobserved to counter what careful observation and analysis show.

As a relative aside, another issue brought forth by Yoo et al. (2005) impinges on the effect of fossils with ambiguous characters on local parts of a consensus tree, which is a well-known phenomenon (Nixon & Wheeler, 1992; Nixon, 1996). Yoo et al. (2005: 699) apparently misinterpret deresolution due to multiple placements of a fossil with actual clade conflict and misinterpret the consensus tree presented by Gandolfo et al. (2004): "However, Gandolfo et al.'s topology disagrees with the morphological analysis of Les et al. (1999), who found strong support for *Victoria* and *Euryale*, consistent with many previous inferences of relationship in Nymphaeaceae; this sister-group relationship is not evident in Gandolfo et al.'s tree."

Apparently, Yoo et al. (2005) misunderstand polytomies—an unresolved polytomy is not in topological disagreement with any dichotomy that it implies—well known in systematics theory and the basis for creation and interpretation of various kinds of consensus trees (see Nixon & Carpenter, 1996, for a complete discussion of these issues). In fact, the trees found by Gandolfo et al. are identical to those reported by Les et al. (1999) when the fossil *Microvictoria* is pruned (removed after the analysis), with high support for the *Victoria–Euryale* clade. The fact that *Microvictoria* floats (Nixon & Wheeler, 1992) within this clade does not mean that it reduces support for *Victoria–Euryale*, which always have exactly the same relationship to other extant Nymphaeales in all most parsimonious trees (Gandolfo et al., 2004). It merely means that given these data, *Microvictoria* is ambiguously placed, sometimes as a sister to *Victoria*, sometimes to both *Victoria* and *Euryale*. The consensus is thus unresolved in this area. If viewing the component most parsimonious trees, when only extant taxa are considered, *Victoria* and *Euryale* always form a monophyletic group of two taxa, as in Les et al. (1999) and as stated by Gandolfo et al. (2004). Note also that Gandolfo et al. (2004), because they restricted their analysis to the original Les et al. (1999) matrix, did not include the feature of prickles on the outside of the flower and pedicel—a synapomorphy of the *Victoria–Euryale* clade that would exclude *Microvictoria* from that clade—since the floral cup of *Microvictoria* is covered externally with bracts, not prickles.

Other molecular-dating analyses, using much larger data sets and different fossils to calibrate their analyses, have found results that are in conflict with Yoo et al. (2005). Wikström et al. (2001) did an NPRS analysis on a much larger and more diverse angiosperm data set (567 taxa vs. 16 taxa, also with three genes), using putatively fagaceous Cretaceous fossils to calibrate one of the 567-taxon three-gene trees (Soltis et al., 2000). The results presented by Wikström et al. (2001) suggest an age for the Nymphaeales of 171–153 Ma, with divergence of the extant crown group occurring 144–111 Ma (consistent with, and considerably older than the fossil *Microvictoria*, aged at ca. 90 Ma). Given these results, one might suggest that perhaps there is a problem with the Yoo et al. (2005) paper, not the identification of *Microvictoria*. Wikström et al. (2001: 2211) also note the phenomenon of discovering ever-older examples of important extant clades of angiosperms: "...fossils considered to be members of derived angiosperm lineages are being documented from increasingly older geological deposits. Crepet and Nixon (1998), for example, documented Clusiaceae from Turonian (90–88 Myr) deposits of New Jersey.... The presence of such derived groups in Cenomanian–Campanian deposits implies either that we have underestimated the rapid and explosive nature of the angiosperm diversification or that cladogenesis in basal angiosperms took place considerably earlier than fossil-based estimates have indicated." Note that, faced with a discrepancy between fossils and molecular dating (a very common occurrence), Wikström et al. (2001) provide two scientific, reasonable alternative explanations, and neither reject the fossils nor the molecular-dating methods.

Conclusions

Homogeneous rates of sequence evolution (a molecular clock) seem to be rare at best. In the example provided by Yoo et al. (2005), significant heterogeneity of rates was detected. This is consistent with many other studies and, in fact, is often easily seen in cladograms (whether derived by likelihood or parsimony) when branch lengths are displayed. Methods such as NPRS and PL merely move the molecular clock assumption to within-clade calculations (by smoothing rates locally) and are susceptible to poor calibration—using one or a few fossils from clades that are not representative of rates in other parts of the tree. If rates are very slow near the calibration fossil, one will come up with overestimates of ages in other parts of the tree; if rates are very fast, one will come up with underestimates. The possibility that two different fossils from different parts of a clade could provide very different estimates on the same trees is not at all surprising. Because of this, if one wishes to use molecular-dating methods, one should utilize as many different fossils as possible (assuming they are well identified and phylogenetically placed); fossils might be used together or in separate analyses to compare how disparate the results become. The fact that a particular fossil provides different rate estimations than other fossils no more impugns the identification of the fossil than it does the assumption of clocklike rates near the fossil node that are representative of rates in the tree as a whole. Because fossils are based on direct observations and rates cannot be observed, one must favor fossil identification over rate assumptions based on a model.

By smoothing vertically through clades, NPRS and related methods explicitly apply a local molecular clock where possible. Rate smoothing, by isolating chunks of the tree, is biased against rapid diversification followed by slow evolutionary rates—i.e., punctuated equilibrium, a popular concept based on observation of actual fossils through the record. The issue here is not whether *Microvictoria* is or is not a

member of the crown group of Nymphaeaceae/Nymphaeales. The issue goes to the core of how we do science. Yoo et al. (2005) rejected fossil identifications based on careful morphological work and cladistic analyses and presented only two alternative explanations: that the method is wrong ("methods of estimating divergence times from molecular data are highly dubious" [Yoo et al., 2005: 700]), or the angiosperms are unreasonably old. These prima facie false premises were then used to question the placement of the nymphaealean fossils, which, as noted above, were consistent with larger, more robust analyses (Wikström et al., 2001).

Because Yoo et al. (2005) did not supply an alternative morphological analysis of *Microvictoria* but instead created a scenario that some unspecified lineage may have existed with the same characters as modern Nymphaeaceae, their conclusion that *Microvictoria* (and the fossil from Friis et al., 2001) are doubtfully part of the Nymphaeaceae crown clade cannot be evaluated. The Yoo et al. (2005) paper is useful for pointing out what not to do in terms of fossil data and molecular dating. Because rates may (and do) change radically within lineages, any particular fossil may be a poor choice for calibration of an entire tree. Calibration with *Microvictoria* produces one answer; calibration with fagaceous fossils on a larger (better taxon-sampled) tree produces an entirely different result. This is not surprising.

The conclusion presented here is simple: it is dangerous, and verificationist, to accept or reject fossils based on whether they fit a preconceived notion of the correct results. It is likely that with almost any data set, a fossil could be found to calibrate a molecular-dating model that would provide a wide range of dates for a particular node of interest. If the results become the primary consideration in deciding which fossils should be used, the scientific endeavor is abandoned and the study becomes an exercise in verification. Because of these constraints, the most casually studied fossil by a competent paleobotanist, if placed in phylogenetic context with explicit characters, should be favored in every case over what might be termed a secondary analysis—and rejecting direct observation based on an interpretation of history constructed on a model.

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HIDE AND GO SEEK: WHAT DOES PRESENCE MEAN IN THE FOSSIL RECORD?

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ABSTRACT

Our efforts to reconstruct accurate, complete records of events in vegetation history and in plant evolutionary history depend on accuracy in dating sediments, interpretation of structures preserved, reconstruction of whole organisms or communities from the preserved material, and interpretation of the interaction between past abundance and fossil presence. This contribution examines the interaction between past abundance of a target plant and the probability of retrieval of that species in the fossil record. By examining records of recolonization in volcanic areas, records of invasive species spread, succession in disturbed habitats, and historical migration patterns, we can provide estimates of the likelihood of appearance in the potential fossil record of newly evolved and reasonably successful species. The lag in discovery, recognition, and publication of a fossil as an important representative of a critical clade is also evaluated and is highlighted as a more important constraint on the use of fossils in testing evolutionary and ecological hypotheses than the recolonization rate. The lag between discovery and publication is particularly relevant in areas of the modern world where fossil plant–bearing deposits are either rare or inaccessible. Greater awareness of the density and reliability of the plant record should allow evolutionary biologists and paleoecologists to bracket not only time intervals but also geographic regions where the fossil record can be interpreted largely at face value. At the same time, more effort should be focused on intense collecting efforts and training in areas where fossil deposits are potentially present, but poorly collected and evaluated.

Key words: Invasive species, paleobotany, plant evolution, stratigraphic resolution, taphonomy.

The fossil record has a pivotal role in retelling the story of plant evolution. Research into the molecular basis for development of plant organs and the application of DNA amplification and sequencing techniques to outline plant phylogenies interfaces with direct evidence from the fossil record. The fossil record has the potential to place time constraints on models of lineage evolution, diversification, specific morphological traits and combinations, as well as to clarify the longevity of both traits and characters (Rothwell, 1999; Frohlich & Parker, 2000; Magallón & Sanderson, 2001; Pryer et al., 2001; Crepet et al., 2004; Davies et al., 2004; Soltis & Soltis, 2004; Anderson et al., 2005).

These new and exciting applications require increased rigor and specificity from our reading of the fossil plant record. Era-level dates, formation-level stratigraphic placements, and nonquantitative descriptions and reconstructions are no longer sufficient. Similarly, superficial readings of the paleontological literature are no longer likely to adequately test evolutionary hypotheses. Original provenance of the plants in a community, as well as the current and past location of the geological crust on which they are deposited, have become much more important in research on plants in the geological record.

However, a caveat can almost always be raised: the fossil record is incomplete. Ecological and evolutionary processes often operate over short time intervals, and, therefore, the incomplete fossil record is best used with, or even replaced by, other types of data. So what should the interpretation be when, at arm's length, the fossil record indicates that many of the significant and defining features of the angiosperms appeared virtually simultaneously (Darwin, 1903; Crane et al., 1995; Magallón et al., 1999; Crepet, 2000; Anderson et al., 2005; Davis et al., 2005; Friis et al., 2006)? Even when the sequence of phylogenetic events is addressed at a finer scale, such as within angiosperm subgroups (Saxifragales, Malpighiales), the density of fossil data and molecular analyses available point to the rapid evolution of currently diverse and widespread lineages in the Albian and Cenomanian (Fishbein et al., 2001; Davis et al., 2005). With these relatively well-constrained studies, confidence intervals on individual branching events can range from seven to 28 million years. In some cases, fossil evidence applied to dating molecularly based phylogenies rests on a single occurrence of a

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single plant organ from few sedimentary outcrops (e.g., Wikström et al., 2001; Bremer et al., 2004; Crayn et al., 2006). While we should take seriously the verified presence of a taxon as excellent data, regardless of its uniqueness, neither the absence of a lineage nor the gap between occurrences is typically so carefully weighed. This biased approach to the fossil record is analogous to a hypothetical example of a molecular biologist extracting DNA from a 1 × 1 cm piece of tissue from a single mature leaf from a single forest in Thailand and characterizing the phylogenetic position of an entire family of angiosperms based on a sequence of 850 bp from that leaf. More data are potentially available, but for logistical reasons they just are not collected, processed, and applied.

Treatment of the obvious gaps in the fossil record can take two routes. Either gaps are ignored, reporting only what we know now, or gaps are acknowledged and the weakness of the record highlighted as an area for future research. The second route does not need to declare defeat but, rather, to state the uncertainty associated with the lack of sequential, consistent fossil occurrences. A parallel situation occurs with the enthusiastic reports of increased crop yield from radically elevated CO_2 concentrations based on small enclosures. The conclusion is that increased CO_2 may be a positive result for humans. Simultaneously, however, open-grown crop plants in elevated CO_2 sites (Free Air CO_2 Enrichment [FACE]) are only marginally higher than in control sites (Long et al., 2006). Knowledge of the data from open field experiments fills a gap that otherwise would provide for misplaced optimism under increased CO_2 for crop yields (Tubiello et al., 2007). In a similar fashion, this contribution is intended to focus attention on the gaps in the fossil record as a means of encouraging investigations that will improve our understanding of the limits of those gaps.

An outline of the events relevant to fossilization of plants is first presented, starting with the evolution of a new species and ending with the use of that fossil species in a phylogenetic or morpho-developmental context (Fig. 1). Each step involves some modification of the original abundance, morphology, and likelihood of recovery of the plant species. Each has the potential to distort subsequent interpretations. The uncertainty introduced by each step in this chain of events should theoretically be quantifiable and, thus, provide us with an estimate of potential error. Two of these steps are highlighted (bold print, Fig. 1) in the remainder of this contribution, relative to their importance in interpretation of the quality of the reported fossil record.

Steps in the fossilization of fossil plants have been addressed particularly carefully by Spicer (1981), Scheihing and Pfefferkorn (1984), Ferguson (1985), Gastaldo et al. (1987), Gastaldo (1988, 1994), Burnham (1989, 1993), Greenwood (1991), Burnham et al. (1992), and van der Burgh (1994). In each of these studies, the steps in the fossilization process are outlined for slightly different reasons, and thus slightly different interpretations emerge. A frequent casualty of the fossilization process is the qualitative and quantitative correspondence between the source forest and the plant debris incorporated into the fossil record. As fossilization proceeds, selective bias toward thicker and more abundant leaves most often results, even if these are not the most common in the source forests. In addition, small-statured species (herbs, shrubs, lianas) are reduced in abundance, relative to large-statured species like trees (Scheihing, 1980; Burnham, 1997). Furthermore, species with distinctive and well-preserved morphological features are most readily identified by paleobotanists, and species with a preference for moist, soggy, lacustrine, or riparian habitats are favored during fossilization over those with high abundance in dry or mesic habitats (Greb et al., 2006). These are important aspects of interpreting the fossil record, and prior studies have suggested the number of specimens needed for accurate interpretations, the environments most likely to preserve an accurate record of the paleovegetation, and the effect of differential degradation of plant species and plant organs.

This set of steps, from the first ecological and evolutionary appearance of a plant group to its use by humans in describing evolutionary history, is a critical pathway in interpretation and fidelity of the fossil record. Each factor can vary with geographic region, climatic regime, type and continuity of sedimentation, plant habit and abundance, and distinctiveness of the species. Any blanket statements about the character of the fossil plant record and its fidelity are clearly distillations of the variability inherent in the fossil record. In general, if the fossil plant record is to be used most reliably, records should be based on large-bodied, abundant, perennial wetland species, whose reproduction is frequent and includes both sexual and asexual reproductive organs. Even so, we should expect large gaps in the fossil record due to periodic loss of habitat, lack of sedimentation, and erosional activity.

Two fossilization steps are addressed here (Fig. 1) to clarify the constraints and advantages of applying the plant fossil record to evolutionary history. First addressed is **abundance increase** (increase in population density and extent), which asks the question: how long does it take from simple presence as a species to abundance of the species with biomass large enough to have a high likelihood of represen-

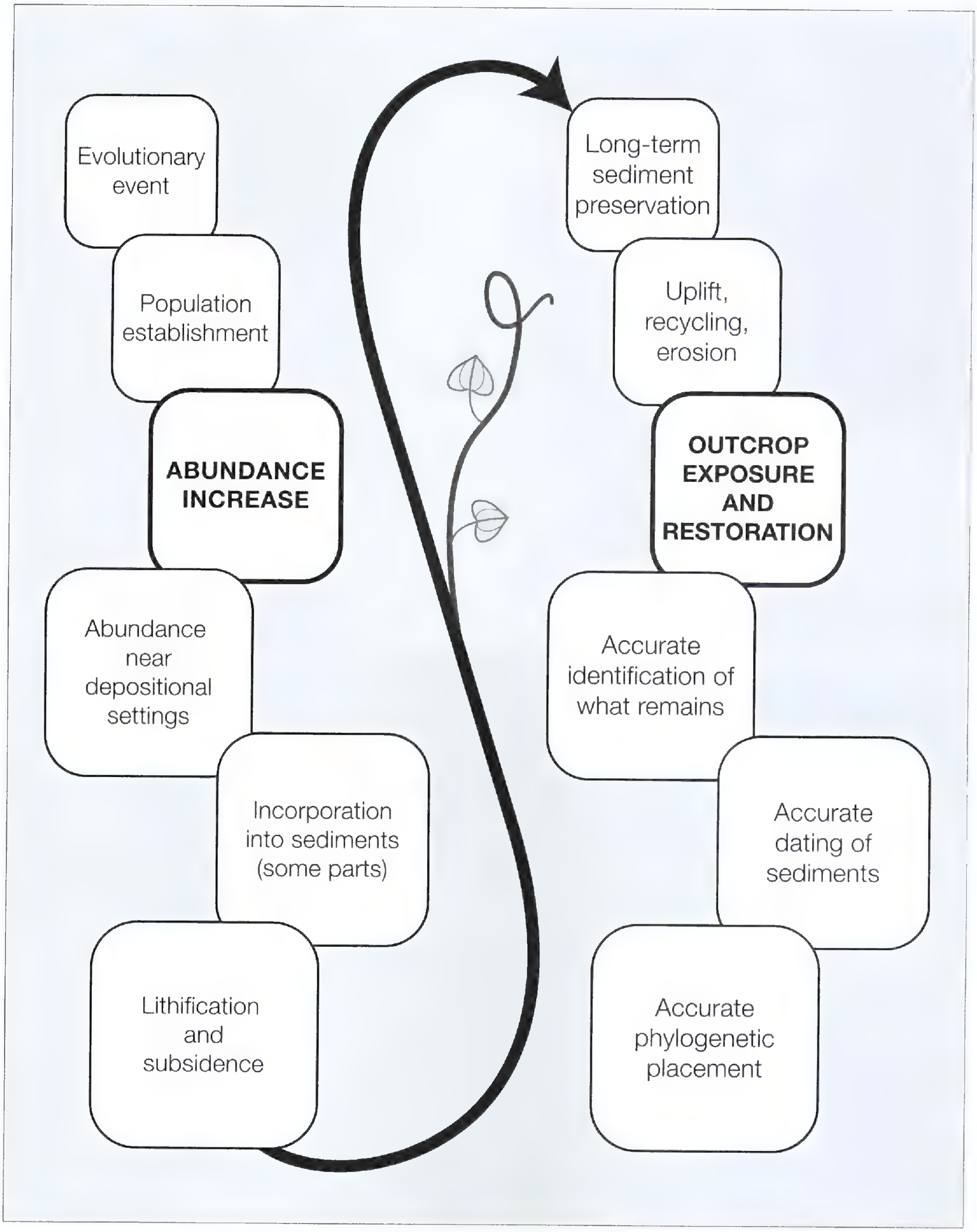

Figure 1. Steps involved in fossilization of a newly evolved plant species. Each step involves some modification of the original plant form, population size and extent, abundance, or relationship to associated organisms.

tation in the fossil record? Then, several important steps are bypassed to address **outcrop exposure and exploration**, both of which are important in controlling the quality of interpretations made from the plant fossil record. This second step poses the question: are appropriate fossil-bearing deposits well represented and sufficiently studied in areas proposed as cradles of evolutionary events? A final note is added that opens the door on issues surrounding long-term sediment preservation.

Step One: Population Expansion and Migration. How Long Does It Take for Populations to Expand to Contribute to the Fossil Record?

Following the evolutionary establishment of a new plant species, it has been proposed that there is a substantial lag time before species have the potential to contribute to the fossil record, either because new species tend to be geographically restricted, or because they tend to be relatively rare in comparison to their longer-established relatives. This proposition, often linked to the idea of ghost lineages and added to (justifiable) suspected lack of taxonomic identification, is frequently cited in the plant evolution literature. This leads to the conclusion that the lag time between the evolution of the first angiosperm and its appearance in the fossil record can create an apparent explosion of angiosperm forms at its first appearance (Frohlich & Parker, 2000; Qiu et al., 2000). Interestingly, a gap in the record is rarely cited for the similarly radical innovations of grasses, where a more stately progression is generally easily described (Jacobs et al., 1999; Jacobs, 2004; Stromberg, 2004; Prasad et al., 2005). Although the lag time is rarely explicitly stated, for the explanation to fit the fossil record, the ecologically controlled lag times would need to be on the order of one to five million years to cause much discernable difference in analysis. Currently, this potential lag time is of critical importance in using fossil plants to date the appearance of higher-order clades (e.g., families, orders) and calibration of phylogenies based on molecular sequence data.

Although this argument has a great deal of potential validity, it has never been seriously tested using modern plant data from analogous evolutionary and ecological scenarios. Here, four ecological scenarios are examined in which expansion to large population sizes is used as a test of the lag time explanation for apparent gaps in the fossil record. The argument proposed is that if we evaluate settings under which plants colonize and expand, we can use the time required for colonization and expansion of large population sizes as a measure of the minimum lag time expected in the geological record. It is explicitly acknowledged that data are presented from success stories (i.e., species with capability for expansion, dispersal, and population longevity that may not be typical for the stem group of many plant groups). Therefore, data presented are the minimum time required for colonization and expansion of a newly evolved species. On the other hand, it could be argued that it is just those species with strong expansion capabilities that will ultimately form the stem group of a successful plant group. Either way, it is prudent to view these time frames as minimum estimates.

Scenario 1: Post-Volcanic Colonization

Volcanic environments present a setting in which we can follow succession using a clearly defined starting date. Numerous volcanic eruptions over the past 1000 years are sufficiently studied to provide descriptive data on vegetative colonization. A massive eruption in Papua New Guinea (Long Island) is dated at 1645 and is estimated to be about the magnitude of the island volcanic eruption on well-known Krakatau. Some 350 years later, 273 species of seed plants, including 31 species of the genus *Ficus* L. alone, plus 32 pteridophytes are present (Harrison et al., 2001; Shanahan et al., 2001). Motmot, a caldera island established as a habitable island in 1968, had established *Ficus* seedlings within only three years of volcanic activity cessation (Shanahan et al., 2001). Even so, this level of recolonization is species-poor compared to the rich flora of nearby Papua New Guinea. The slow colonization has been attributed to the dry volcanic soils relative to the richer flora of Krakatau, which has a similar island setting and shorter eruptive history.

In comparison, the massive eruption in 1883 that destroyed vegetation (and habitations) on Krakatau left behind fertile ash soils in a humid tropical environment, both of which promoted rapid and diverse colonization. Only 100 years after the eruption, over 400 species had colonized (Whittaker et al., 1997), including some 100 species of climbing plants. Whittaker and colleagues propose that the island's flora is still disharmonic but "becoming less so" just 104 years following the devastation of the original vegetation (Whittaker et al., 1989; Bush et al., 1995; Thornton et al., 1996; Whittaker et al., 1997: 1671).

Volcanic environments can be rich in nutrients but relatively slow to accept colonizing species because the mantle of tephra and ash blankets the relatively more nutrient-rich debris flows and pre-volcanic soils. Because of high dispersal capabilities, ruderal species are generally the quickest to colonize (del Moral & Bliss, 1993). On Mount Lassen in California, forest trees may not have begun colonization until as much as 30 years after the cessation of eruptive activities (Kroh et al., 2000). Although colonization in volcanic environments can be slow, growth rates can accelerate once successful establishment has taken place, with many tree species reaching maturity within 20 years. On Mount St. Helens (Washington State, U.S.A.), interactions between site amelioration, dispersal ability, and colonizing ability prevent any concrete predictions on reassembly of communities (del Moral et al., 2005). However, species richness started to approach equivalence across all habitats surveyed by 20 years following the major eruption of 1980. In

contrast, percentage cover was most rapidly increasing in safe sites and strongly ameliorated sites. Recovery of volcanic environments is clearly a slow process and is highly subject to the species attributes and environmental changes of the specific area. Vegetation recovery on Mount St. Helens 22 years after eruption indicates that stochastic processes are still in effect; deterministic processes have not yet taken over (del Moral & Lacher, 2005).

With relatively sparse data on long-term events in volcanic environments, we can still propose that in the absence of repeated events, even 2000 years should be sufficient for species in plant communities to expand to fossilizable population sizes. In the Neogene geological record of the western United States, it is clear that frequent disturbances can reset the ecological setting on long-term succession, as shown by Taggart and Cross (1990) for the Succor Creek and Trapper Creek floras.

SCENARIO 2: INVASIVE SPECIES INTRODUCTIONS

Another approach to estimating the rate at which new species can become abundant enough to contribute to the fossil record is to determine the rate at which introduced species spread across a new landscape. Knowing the date of the first documented arrival of a species (based on herbarium specimens, first cultivation dates, or vegetation surveys) provides a proxy for a new species origination. Literature on introduced and invasive species is reviewed here with the following question in mind: How long does it take to become a pest (common enough to become a fossil)?

Published literature is rich with reports of the impact and importance of invasive species, so this review is not intended to be exhaustive, but rather a sampling of life forms and phylogenetic diversity covered by the literature. In addition, summary ideas on invasive plants are extracted, relevant to the arguments here. Although attributes of species can help predict their propensity to be invasive, it is generally agreed that predictions are statistically based and individual cases are normally unpredictable (Kowarik, 1995; Rejmánek & Richardson, 1996; Williamson & Fitter, 1996; Kolar & Lodge, 2001; Hallet, 2006). A temporal framework for this discussion was provided by Usher (1988), who remarked that after 1000 years, it is virtually impossible to distinguish exotic from native plant species.

Based on intentionally imported species, a rule has been proposed that one of 10 species is likely to become naturalized once imported. Of those, one of 10 is likely to become a pest (Kowarik, 1995; Williamson & Fitter, 1996). Exceptions are important because crop plants are very likely to become naturalized (71 of 75 surveyed; Williamson & Fitter, 1996) and of 235 species introduced for horticulture, 82% have been found naturalizing outside of cultivation (Reichard, 1997). Still, the important step to pest status is quite low for crop species, yielding a one in 100 chance of rising to pest status for most imported crop species. Interestingly, this value is similar for Hawaiian plants (90 in 900 of naturalized invasive plants are threats to ecosystems; Loope, 1998), which have gained a great deal of attention because of their island setting.

An abbreviated list of invasive plants for which dates of introduction and approximate dates of pest status are readily available is presented in Table 1. This compilation does not include crop plants or plants that are only invasive in island ecosystems. Sampled are a range of life forms (trees, herbs, lianas, shrubs, aquatic plants) and a range of phylogenetic diversity (ferns, conifers, dicotyledonous angiosperms, and monocotyledonous angiosperms). At the species level, the results are astonishing because some introduced species rapidly rise to pest status. At a community-floristic level, Henry and Scott (1981) reported that 29% of the plant species in Illinois were documented aliens, and 13% of all woody species were aliens as of their study date. These figures are in line with figures reported from other preserved areas of the United States (Pimental et al., 2000: 26% in Great Smoky Mountain National Park; Yost, 1991: 36% alien to the New York State flora).

Table 1 suggests that if a species has a propensity to become abundant, it can rise to abundance in less than 500 years in almost all cases studied. There is a surprising amount of consensus on what combination of attributes most predispose introduced plant species to become invasive. Despite the statement by a National Academy Committee (Committee, 2002) that "The rate of immigration of plants and arthropods via *natural* [italics mine] means appears so low as to cause little concern about the arrival of detrimental species," it is likely that on a scale of 1000 years, this natural immigration would be of note. It is this longer scale we are concerned with here. Similarly, while plant traits are important, the complementary view that the disassociation of plants from their coevolved parasites and herbivores is also an important aspect of current plant invasiveness (Lake & Leishman, 2004; Hallett, 2006). Newly evolved species may have few parasites early in their evolutionary history, which would only add velocity to the rate of expansion. I emphasize here that the ecological literature clearly treats lag times as real and they certainly are so on an ecological time scale, but the time lags mentioned in the ecological literature are almost always less than 2000 years (Sakai et al., 2001; Crooks, 2005). In a particularly

Table 1. Examples of selected invasive species for which the following can be determined: place of origin, place of invasive record, time to recognized pest status, and literature source.

Genus and species	Family	Place of origin	Place of invasion (first appearance date)	Time to pest status (years)	Source(s)
Acer platanoides L.	Aceraceae	continental Europe and the Caucasus	MA, NJ, DE (introduced ca. 1775)	< 200	Webb & Kaunzinger, 1993
Schinus terebinthifolius Raddi	Anacardiaceae	South America (Brazil, Argentina, Paraguay)	Central America, Bahamas, Caribbean islands, FL, Mediterranean Europe, North and South Africa, China, South and Southeast Asia, Australia, Pacific islands (1832 or 1842–1849)	70	Morton, 1978; Ferriter, 1997; Williams et al., 2005
Vincetoxicum rossicum (Kleop.) Barbar.	Apocynaceae	Ukraine and Russia	northeastern and midwestern America (1890)	100	Sheeley & Raynal, 1996
Ambrosia artemisiifolia L.	Asteraceae	America	France (1865–1880; possibly 1681?)	15	Dessaint et al., 2005; Pederson et al., 2005
Solidago altissima L.	Asteraceae	North America	Europe (1750?)	250	Weber, 1998, 2001
Solidago canadensis L.	Asteraceae	North America	China (1935) to Shanghai	70	Weber, 2001; Dong et al., 2006a, b
Berberis thunbergii DC.	Berberidaceae	central and southern Japan	NJ to NH, west ND and SD; introduced to Arnold Arboretum (ca. 1870)	100	Ehrenfeld, 1997, 1999
Butomus umbellatus L.	Butomaceae	mainland Europe, U.K., Ireland, temperate western Asia	northeastern North America (1897)	< 100	Tutin et al., 1980; Brown & Eckert, 2005; Kliber & Eckert, 2005
Lonicera japonica Thunb.	Caprifoliaceae	Asia	eastern U.S.A. (ca. 1860)	< 125	Schierenbeck, 2004; Yates et al., 2004; Yurkonis & Meiners, 2004
Celastrus orbiculatus Thunb.	Celastraceae	southeastern Asia	eastern U.S.A. (1860)	90	McNab & Loftis, 2002; Ellsworth et al., 2004; Leicht & Silander, 2006
Bryonia alba L.	Cucurbitaceae	central and eastern Europe		50	Novak & Mack, 1995, 2000
Sphaeropteris cooperi (F. Muell.) R. M. Tryon [syn. *Cyathea cooperi* (F. Muell.) Domin.]	Cyatheaceae	Australia	HI (1950s)	40	Medeiros et al., 1992; Durand & Goldstein, 2001
Elaeagnus umbellata Thunb. ex Murray	Eleagnaceae	Japan, China, Korea	IL (and throughout the northeastern U.S.A.)	21	Ebinger, 1983; Yates et al., 2004
Sapium sebiferum (L.) Roxb.	Euphorbiaceae	subtropical China	GA (1772)	ca. 200	Bruce et al., 1997; Barrilleaux & Grace, 2000; Siemann & Rogers, 2001

Table 1. Continued.

Genus and species	Family	Place of origin	Place of invasion (first appearance date)	Time to pest status (years)	Source(s)
Pueraria lobata (Willd.) Ohwi	Fabaceae	Asia	Philadelphia, FL, southeastern U.S.A. (1876)	75	Miller & Edwards, 1982; Miller, 1985; Mitich, 2000; Pappert et al., 2000; Lembke, 2001
Lygodium microphyllum (Cav.) R. Br.	Lygodiaceae	tropical Southeast Asia, Africa, Australia	southern FL (1958)	20	Nauman & Austin, 1978; Pemberton & Ferriter, 1998; Dougherty, 2003; Lott et al., 2003; Volin et al., 2004
Syzygium jambos (L.) Alston	Myrtaceae	Southeast Asia	Puerto Rico (1821)	115	Brown et al., 2006
Psidium guajava L.	Myrtaceae	tropical America	Fiji, Namibia, Galapagos (1870)	120	Partridge, 1979
Pinus radiata D. Don	Pinaceae	North America, 4000 ha. on CA coast	Australia, South Africa, and New Zealand (1865), but large-scale planting in 1930	130 max., possibly only 60	Cronk & Fuller, 2001
Spartina alterniflora Loisel.	Poaceae	Gulf of Mexico, east coast of North America	WA (Willapa Bay) (ca. 1905)	90	Stiller & Denton, 1995; Davis, 2005
Polygonum perfoliatum (L.) L.	Polygonaceae	East Asia: Japan, Philippines	OR then PA (1890)	75	Reed, 1979; Oliver, 1996; Kumar, 2005
Rhamnus cathartica L.	Rhamnaceae	Europe	North America (1824)	ca. 100	Gourley, 1985; Heneghan et al., 2004
Paederia foetida L.	Rubiaceae	Japan	FL, NC, TX (ca. 1867)	50	Brown, 1992; Diamond, 1999; Flores, 2003
Salvinia molesta D. S. Mitch.	Salviniaceae	southeast Brazil	Kakadu Natl. Park, Australia; SC (1995); TX (1998); LA to NC (2000)	1	Cowie & Werner, 1993; Jacono & Pitman, 2001; McFarland et al., 2004
Selaginella kraussiana (Kuntze) A. Braun	Selaginellaceae	South Africa, Azores	Neotropics, southern U.S.A., Europe, New Zealand esp., HI, Norfolk Island, Waitakere (?) (1919, New Zealand)	< 80	Wilson, 1996; Roy et al., 2004
Ailanthus altissima (Mill.) Swingle	Simaroubaceae	eastern and central China	U.S.A. (1784); Ontario, Canada (1998)	ca. 100	Meloche & Murphy, 2006

detailed review of lag times, Kowarik (1995) reported a time lag of about 147 years between introduction and invasive status among 182 species studied.

Characteristics commonly cited as beneficial to plants establishing expanding populations across a new habitat or continent, and thus potentially leaving an immediate fossil record, have been addressed frequently in the literature (Baker, 1974; Scott & Panetta, 1993; Pyšek et al., 1995; Rejmánek & Richardson, 1996; Reichard, 1997; Reichard & Hamilton, 1997; Clark et al., 2001; Kolar & Lodge, 2001; Sakai et al., 2001; Lake & Leishman, 2004; Richardson & Rejmánek, 2004; Rejmánek et al., 2005). From these, we can list attributes most likely to be found in plant species with the best potential for an immediate fossil record following evolutionary appearance as follows: vegetative reproduction, including forming thickets with high leaf area; large number of propagules produced and high consistency in production; small seeds and/or propagules with strong adaptations for vertebrate dispersal; short juvenile period; family or genus includes other invasives; broad ecological tolerance (via phenotypic plasticity and/or broad latitudinal range). Species of fossil plants most likely to bear these characteristics are the best candidates for use in reconstructions and paleoclimatic and phylogenetic studies.

SCENARIO 3: SUCCESSIONAL FOREST AND REGENERATION RATES

A third strategy for estimating the rate of expansion of new species is to examine records of regenerating forests in temperate and tropical landscapes. Forests that have been clear-cut for ranching, lumbering, or mining, or devastated by landslides or hurricane winds are ideal settings for estimating rates of regeneration of populations to abundances that can contribute to the fossil record. This scenario is treated here separately from that of volcanic settings largely because of a more rapid rate of regeneration due to plant root and stem stocks and the smaller geographic extent of destruction expected. The two scenarios overlap to some degree.

Again, the literature is rich in case studies of forest regeneration. Only a few of the many studies from temperate, subtropical, and tropical climates are selected for estimation of regeneration rates. This summary is restricted also to the regeneration of woody species, which are the most likely modern analogs to contributors to the fossil record (Ferguson, 1985; Burnham, 1997).

In Neotropical lowland sites (either maritime-influenced or wholly continental), the time of regeneration for heavily disturbed or damaged forests is on the order of 100–400 years (Guariguata & Ostertag, 2001) and even less when disturbance is short or less intense (Table 2). Several authors point to the importance of assessing forest recovery in terms of species composition, basal area, and forest function, rather than simply species richness (e.g., Guariguata & Ostertag, 2001). Accurate data on forest regeneration as much as 200 years after disturbance are very rare, so estimates are often made either by comparison among different-aged forest disturbances or by extrapolation from single forests from much shorter time intervals. A direct assessment of forest regeneration could increase the average time of recovery well beyond 400 years, but it is unlikely that it would extend it as much as one order of magnitude to 4000 years. Significantly, repeated and continual disturbance suppresses total forest regeneration as shown by Rico-Gray and Garcia-Franko (1992) in forests in Yucatán, Mexico. A review of the successional literature for the Neotropics published in 2001 was characteristically noncommittal on any maximum rate of forest regeneration but acknowledged that "the regenerative power of neotropical forests vegetation is clearly high" (Guariguata & Ostertag, 2001: 185).

In temperate forests, data on human clearing are more readily available and can be used as the forest destruction date. In these forests, similar results seem to apply: regeneration is on the order of 100–500 years (Table 2). Regeneration time is potentially longer under continued anthropogenic disturbance. In a review of temperate regeneration under large-scale disturbances, Turner et al. (1998) indicate that distance for dispersal and random processes (lack of predictable competitive hierarchies) are likely to apply but, again, over time scales that are not explicitly stated. Most authors report studies from relatively short time intervals, so their reluctance to commit to full regeneration is understandable. The combination of approaches mentioned below under Scenario 4 (migration rates) adds a dimension improving interpretations of these shorter-scale results.

This brief summary of the literature on regeneration in forested ecosystems in a variety of climates indicates that, again, plant species can and do expand to large populations in remarkably short periods of time, geologically. It would be surprising to detect any gap in the fossil record longer than 1000 years due to a single destructive event. Models of forest succession based on observations in the temperate zone generally employ time periods substantially less than that (e.g., Busing, 1995).

SCENARIO 4: MIGRATION – EXPANSION RATES BASED ON FOSSIL POLLEN LINKED TO HISTORICAL RECORDS

The fourth approach taken here is to apply evidence from pollen in sediments to estimate rates of plant

Table 2. Selected studies indicating woody species' composition time to recovery in tropical and temperate forests (including one bog), ordered north to south.

Site	Climate	Recovery time (yr.): woody species	Type of disturbance	Source(s)
Sweden	temperate ombrotrophic peat mire	> 50	peat harvesting 50 yr. before abandonment	Soro et al., 1999
Boundary Waters Canoe Area (MN), U.S.A.	temperate moist forest	60 yr. for demographic transitions	dramatically reduced fire regimes; 57 yr. of direct observation	Frelich & Reich, 1995
Bolleswood Natural Area (CT), U.S.A.	temperate moist forest	> 50	post-agricultural	Fike & Niering, 1999
Fernow Experimental Forest (WV), U.S.A.	temperate mountainous forest	Structurally similar but unrestored after 100 yr.	logging, followed 20 yr. later by *Castanea* blight	Schuler & Gillespie, 2000
Great Smoky Mountains (TN), U.S.A.	temperate moist forest	ca. 200	abandoned agricultural fields	Clebsch & Busing, 1989
Great Smoky Mountains (NC/TN), U.S.A.	temperate	50–200	small natural gaps	Runkle, 1985
Yucatán, Mexico	lowland dry tropical	much more than 26 (forest not recovered at publication)	agave cultivation	Mizrahi et al., 1997
Dominican Republic	tropical maritime	40	60 yr. of agriculture	Martin et al., 2004
Puerto Rico	tropical maritime	40 (similar to > 80-year-old forest)	abandoned pastures	Aide et al., 1996, 2000
Luquillo, Puerto Rico	tropical maritime	< 100	human clearing and hurricane disturbance	Foster et al., 1999
Sarapiqui, Costa Rica	tropical lowland wet forest	ca. 150: dominants regenerated reach large sizes	5–10 yr. of pasture before abandonment	Guariguata et al., 1997
La Selva, Costa Rica	tropical lowland moist forest	< 440 (longest tree lifespan)	undisturbed forest	Lieberman & Lieberman, 1987
Tropical summary: Nigeria to Thailand to Venezuela	lowland secondary forests	average 80 (75–150)	human disturbance	Brown & Lugo, 1990
Barro Colorado Island, Panama	tropical lowland	50–100	natural river meanders	Knight, 1975; Dewalt et al., 2000; Denslow & Guzman, 2000
Colombia, Venezuela, Upper Río Negro	tropical continental	ca. 80–200	2–3 yr. of intensive agriculture on poor soil	Saldarriaga et al., 1988
Gran Pajonal, Peru	montane tropical rainforest	35 yr. to ca. 300 metric tons; uncut control ca. 600 metric tons	light pasture, 0–3 yr. of crop cultivation	Scott, 1977

species expansion from nascent populations to populations large enough to be recovered in the fossil record. Pollen deposited in lake, pond, estuary, or riverine sediments documents the presence of species in the catchment area. First appearances of plant invaders are used as a proxy for species evolution and employed in combination with historical exploration and land use records to give a minimum estimate for definite representation in the fossil record (at least in wetland areas). Similar to other approaches used here, pollen evidence is a best-case scenario resulting in the most rapid rates. The evidence presented overestimates the appearance in the fossil record of slow-moving taxa or taxa whose pollen is poorly represented in sediments. In addition, pollen is likely to be the first plant organ potentially recognized in sediments but is only rarely diagnostic to species (but see recent advances in Finkelstein et al., 2006). Pollen evidence overestimates the rate at which an average species can spread and be recognized in the fossil record.

Crawford Lake (southern Ontario) is a well-known example of pollen records including Native American and European colonizers (McAndrews, 1988; Jackson, 1997). Records indicate that with the expansion of Native Americans using small-scale cultivation methods, species that are known to favor disturbed habitats, such as *Portulaca oleracea* L. and *Zea mays* L., appeared and expanded. Evidence from a variety of sites (Easter Island; Lindsley Pond, Connecticut) was reviewed by Jackson (1997) and the obvious point is that the appearance of pollen of an invasive or introduced species in sediments is often interpreted as synchronous with the arrival of colonizing humans. This interpretation, while certainly not absolutely accurate, is widespread (Whitehead & Sheehan, 1985) and underscores the speed with which introduced species spread on arrival. Jackson (1997) summarized the rate of spatial expansion being on the order of tens to thousands of kilometers within 5000 years for entire plant communities. Synchronous invasions by *Tsuga* (Endl.) Carrière into several *Pinus* L. forests in North America are documented (Davis et al., 1998), evidence of invasions that appear to have taken place over less than 1000 years. Woods and Davis (1989) document range expansions over periods of 500–2000 years for *Fagus* L. Quite appropriately, data for *Fagus* and *Acer* L. were revisited using the distribution of genetic polymorphisms (McLachlan et al., 2005), showing that rates may not have been as rapid as some have envisioned. While the molecular estimates double the previously accepted migration rates of forest trees, even this doubling of migration rates suggests no major temporal gap between the appearance of new species and their recognition in the fossil record for the majority of the fossil record.

These four approaches to estimating the rate of species expansion following establishment support the idea that it is unlikely that gaps in the fossil record can be explained by an intrinsic lag in population establishment and expansion. Plant species appear to be able to both increase in population size and migrate to almost any area inhabitable by the species on the earth. Data are based on rapidly migrating, expandable species, but there is no intrinsic geological lag due to lack of colonizing or dispersal abilities. Time lags of as much as 2000 years may be expected for some species prior to establishment of large populations, contributing to the fossil record. However, this is not a long period of time relative to the acuity of the fossil record and is particularly short relative to the phylogenetic and morpho-developmental questions applied correctly. It is not likely, therefore, that the rate of species expansion is responsible for observed gaps in the fossil record itself or disharmonic results between molecularly based phylogenies and the sequence of appearance of taxa in the fossil record. Instead, observed or perceived gaps must be explained by other mechanisms, of which there are many.

Step Two: Outcrop Exposure and Exploration. Can the Potential Fossil Record Provide Answers?

The sequence of events leading from species origination to recognition and application of plant fossils includes a step that receives little attention: the potential for discovery of fossil-bearing outcrops and recognition of taxa represented (Fig. 1). This aspect of our understanding of earth's history has received remarkably little direct attention in the past, giving us little hard data with which to estimate the potential error in stratigraphic ages or global distributions. Because my interests have focused on fossil plants from northern South America (Burnham & Graham, 1999; Burnham & Johnson, 2004), I cover this topic in some detail here for relatively young sediments (Cretaceous to Pliocene) in northern South America. The topic deserves much more attention and could be profitably covered continent by continent by specialists. Two questions are addressed using data derived from Cretaceous- through Pliocene-age sediments from literature and accumulated outcrop data: (1) Is the quality of the published fossil record sufficient for constraining the timing of evolutionary events in northern South America? (2) If we knew every plant fossil deposited in every accessible outcrop in northern South America, would that be sufficient for dating phylogenetic trees, mapping past ecological or geographic distributions, or documenting a complete sequence of evolutionary events?

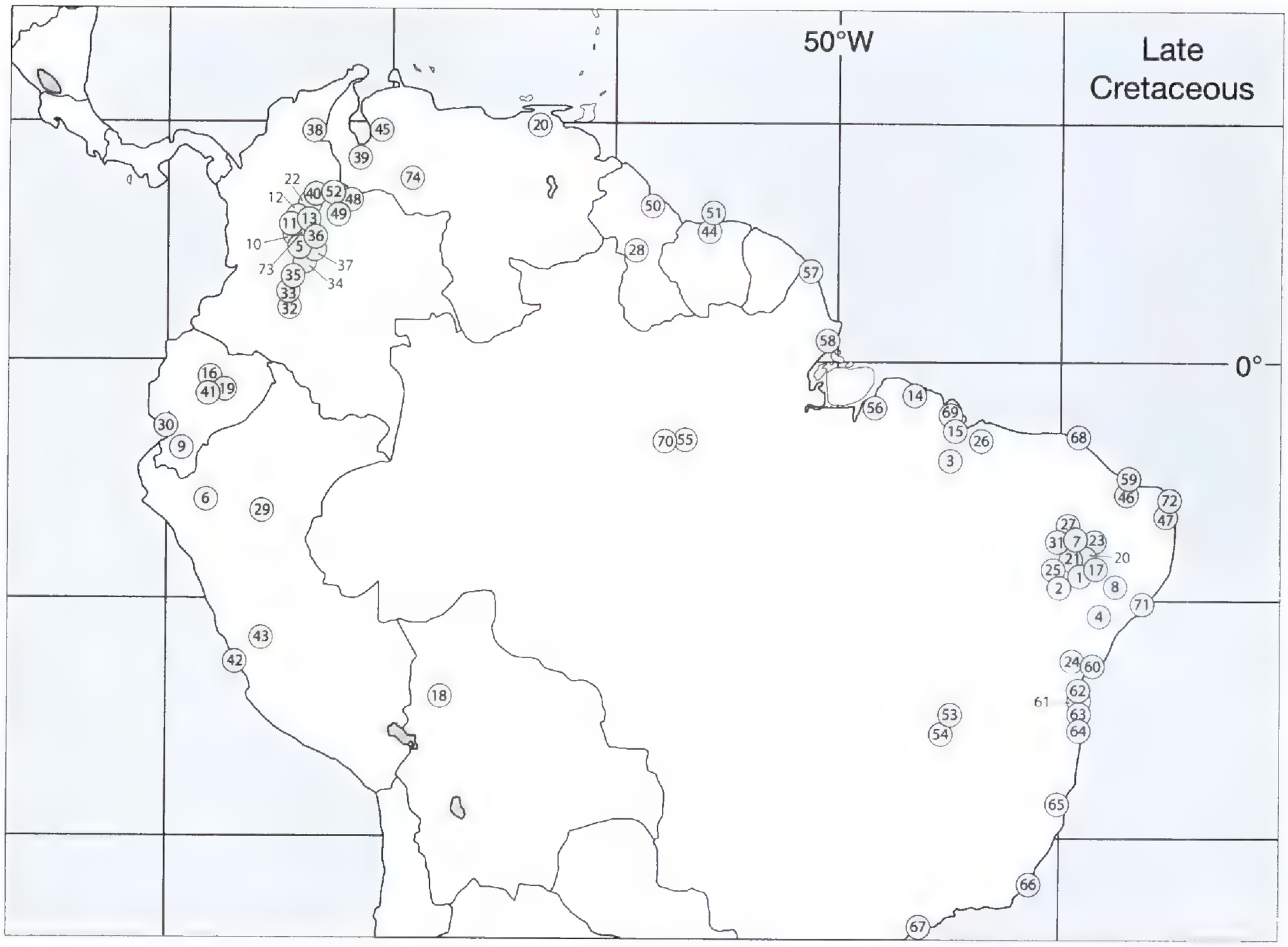

Figure 2. Known late Early and Late Cretaceous–age plant fossil localities in northern South America as of December 2006. Data for localities were extracted from the literature, with each site numbered in a database available from the author. Localities included from Aptian to Maastrichtian.

The Quality of the Published Plant Fossil Record

In 1999, Burnham and Graham published a series of five maps of northern South America, plotting all fossil plant localities known to us from the Late Cretaceous, Paleogene, Miocene, Pliocene, and Quaternary. Collectively, the data appeared rich (Burnham & Graham, 1999: fig. 2), but the 243 fossil localities were scattered over more than 80 million years and over 13,000,000 km^2; thus, the number of localities per time unit or per unit area was really quite small, even compared to our limited understanding of the distribution of modern biodiversity. Moreover, more than a quarter of those localities (28%) represented Quaternary sediments, a tiny fraction (roughly 2%) of the 80 million years of sediment surveyed. In 2006, a resurvey of the paleobotanical and geological literature, published Internet sites, and more complete bibliographic searches turned up a much larger number of sites for each time period evaluated, in addition to seven years of added publication activity (see Table 3). Updated maps are published here for the Late Cretaceous, Paleogene (three subintervals included), Miocene, and Pliocene (Figs. 2–5). Quaternary deposits were not resurveyed in 2006. A total of 332 pre-Quaternary localities are now known (compared to 174 pre-Quaternary localities known in 1999). A comparison of these data sets is presented here as Table 3. Significant in both data sets and from inspection of both series of maps (Figs. 2–5) is the paucity of known plant localities from the Amazon Basin for more than 80 million years (Late Cretaceous through Pliocene; see also Burnham & Graham, 1999; Figs. 2–5). The rocks of the Amazon Basin are complex, and, yet, a large proportion is mapped either as igneous and metamorphic rocks of Precambrian age, or as Pliocene- and Quaternary-age sediments blanketing an unknown base (SIG, 2002). Many of the known fossil plant localities are sediment cores, which typically can only provide palynological data. However, in parts of the Brazilian Amazon, rocks of Cenozoic or Cretaceous age are mapped but poorly studied. These areas represent the sites of greatest changes in density of localities between the 1999 and 2006 surveys of localities, but the large extent of rock that could potentially contribute to the plant fossil record has been underexplored. Improvements on the

Table 3. Comparison of locality numbers per geological period for two literature census dates. Because literature was combed more deeply for the Cretaceous localities in the 2006 survey, the two Cretaceous values differ not only because of the addition of Aptian and Albian localities in the 2006 census, but also because of intensified publication activity and better searching techniques (search engines, online libraries, and software) in 2006. my = million years.

Geological period	Approximate duration (my)	Localities presented in 1999	Localities presented in 2006
Quaternary	1.8	69	Not censused
Pliocene	3.5	35	38
Miocene	18	75	116
Oligocene	10	18	37
Eocene	22	27	44
Paleocene	10	6	23
Late Cretaceous	35 (1999)	13	74
	55 (2006)	Cenomanian to Maastrichtian	Aptian to Maastrichtian
Total localities		174	332
		243: including Quaternary	

known fossil locality maps Burnham and Graham published in 1999 give us hope that further explorations, with this more complete set of known localities, will help close the gaps where they yawn the widest.

The number of fossil data points that contribute to dating recent land plant phylogenies based on molecular evidence is remarkably small and yet is drawn from global sources (Fishbein et al., 2001; Conti et al., 2002; Bremer et al., 2004; Davis et al., 2005; Crayn et al., 2006). South America has the potential to contribute substantially, as demonstrated by the spectacular fossils of early angiosperms and gnetaleans recovered from the Crato Formation in Brazil (Mohr & Eklund, 2003; Rydin et al., 2003) and the diverse angiosperms reported from the Paleocene and Eocene from Colombia (Jaramillo, 2002; Jaramillo et al., 2006) as well as from the Miocene of Ecuador (Burnham, 1995; Burnham & Carranco, 2004; Burnham, unpublished). However, much of the Late Cretaceous and Cenozoic sedimentary rock that is known to exist in South America has not been adequately explored to determine whether it bears fossil plants, much less sustained detailed taxonomic studies. For example, a large expanse of Cretaceous sediment is evident from maps of the adjoining states of Goias, Tocantins, and Bahia, Brazil, but I have not yet found a single reference to fossil plants from this area. Similarly, the geologic map of Bolivia bears indications of appropriately aged sediments, and, yet, this country consistently shows few localities on each of the 2006 maps presented here. These specific examples underscore the importance of gaps in our knowledge of the potentially available fossil record, in contrast to gaps that may be created by lack of deposition, lack of preservation, or erosion of fossiliferous sediments prior to discovery. Some gaps might be filled by data that are still held as proprietary by commercial concerns (oil, gas, and other mining exploration).

The impact of gaps on applications of the fossil record to paleogeography, paleoclimate, phylogenetics, and even ecosystem change is extreme. For example, due to the limited subset of fossil calibration points for phylogenetic trees, the same data can be used to constrain timing for alternative scenarios of plant evolution. Drastically different scenarios can be equally plausible if calibration points are few, or are biased toward specific regions of the world. Similarly, data on paleoclimatic parameters based on fossil plants are limited by known localities that can be analyzed for such data. A single fossil assemblage from a small area can be assumed to be representative of a large geographic area (as is generally assumed), but it can also be atypical, representing a low moist area in a surrounding nondepositional, dry area (Burnham et al., 2001; Kowalski & Dilcher, 2003). Interpolation of paleoclimatic points between sites that are more closely spaced is obviously preferred. Lack of intensive collection and documentation of fossils in certain geographic areas will severely bias paleogeographic reconstructions of species distributions.

In contrast to the promising results from the first step of the fossilization process presented here (Abundance Increase; Fig. 1), in some parts of the world and for some time periods, the fossil record should be read very cautiously. Presence of a taxon in the fossil record is a known anchor for evolutionary events, but such data cannot be used to weight particular areas or time periods if sufficient geological and paleobotanical exploration has not been carried out elsewhere (see, for example, the distribution of localities in fig. 2 of Gingerich, 2006). A recent

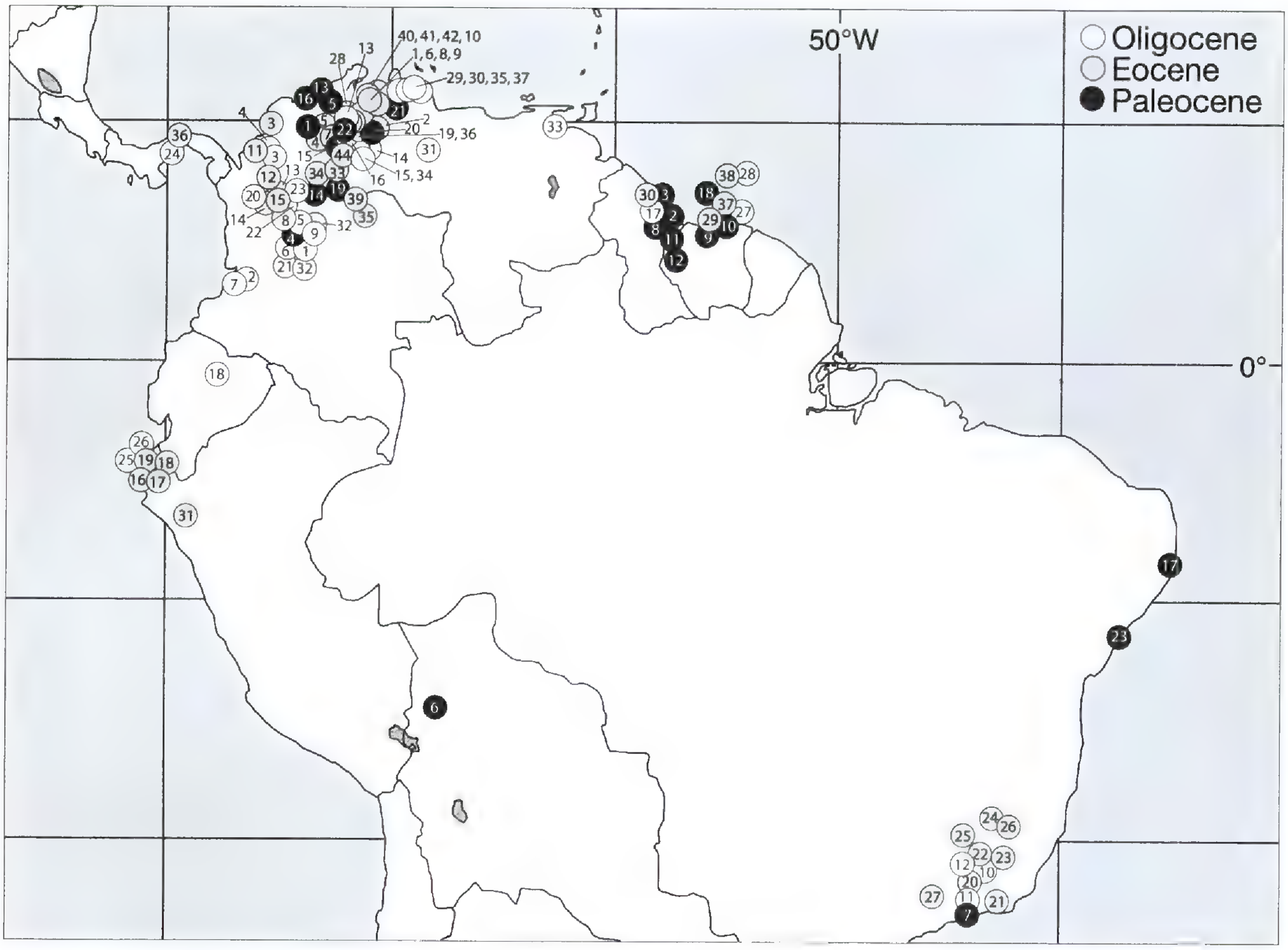

Figure 3. Known Paleogene-age plant fossil localities in northern South America as of December 2006. Data for localities were extracted from the literature, with each site numbered in a database available from the author. Localities are coded to show age assignment to Paleocene, Eocene, or Oligocene.

extensive review of the Malvoid pollen record (Krutzsch, 2004) proposed evolutionary and migration scenarios based on worldwide pollen records. While the review is complete in terms of published information, we have to wonder how much of the scenarios might be altered with just two additional records of Malvoid pollen from South America. The absence of evidence in unexplored areas needs to be recognized as just that.

Can the Potential Fossil Record Answer Our Questions?

The second question posed above is potentially unanswerable because of our lack of understanding of global depositional patterns over time: if we knew the identity and morphology of every fossil deposited in every accessible outcrop, would that be sufficient for confirming the validity of dates for phylogenetic trees or for confirming evolutionary events?

While we can more intensively sample areas of the earth that are poorly represented by fossils of particular taxonomic groups and ages (addressed in the preceding paragraphs), a more substantial issue lurks within the deposition and preservation of the sedimentary rock record itself. Ronov and colleagues (Ronov et al., 1979, 1980; Ronov, 1994) painstakingly measured the area of rock outcrop for epochs over the past 543 million years. These monumental efforts were hampered at the time by the same kinds of gaps in our knowledge of surficial geology in remote areas that we have today. Nevertheless, the work demonstrated (Ronov et al., 1980) that within each geological period from the Lower Permian to the present, the proportion of rock volume composed of coal-bearing rocks (the most likely to preserve plant fossils) is between 0.3% and 8.1% (a high of 11.2% is recorded in the Carboniferous period). If we add all other sedimentary rocks that could potentially incorporate plant remains (but probably do not, such as coarser river sediments) to those coal-bearing sediments, we get a maximum of 16%–30% of the rocks preserved from any geological period, which have the potential to bear identifiable plant remains. Of course this is highly variable, temporally.

This result is sobering because this may mean that mapped geological exposures of any specified time period have, on average, less than a 30% chance of

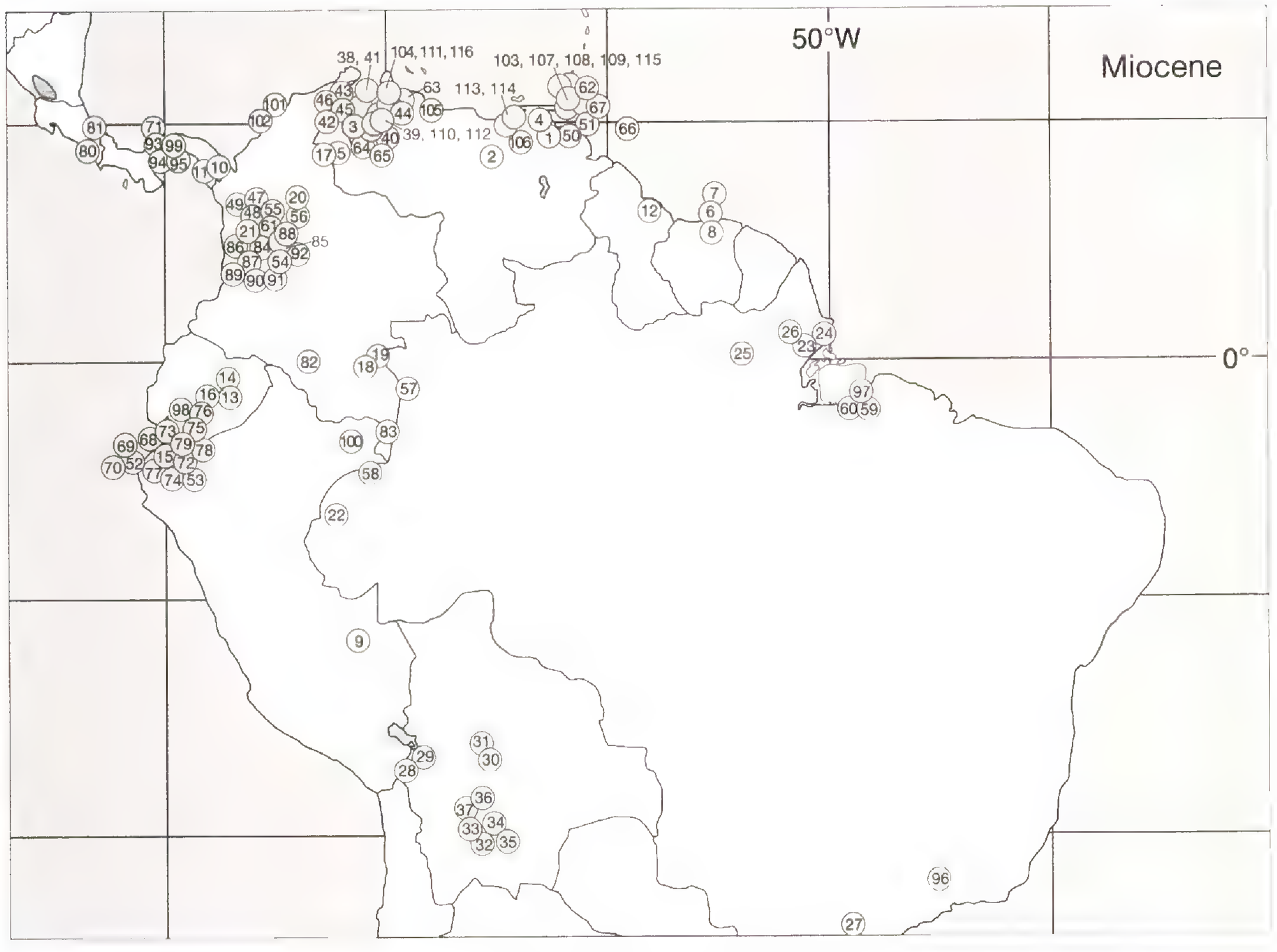

Figure 4. Known Miocene-age plant fossil localities in northern South America as of December 2006. Data for localities were extracted from the literature, with each site numbered in a database available from the author.

yielding rocks that potentially bear plant remains. What is of even more concern is a concept recently cogently explained by Peters (2005) that the potential for sedimentary deposition *and* preservation is strongly controlled by continental position and, in particular, sea-level change. Peters (2005, 2006) employed the COSUNA (Correlation of Stratigraphic Units of North America) charts, which are based on some of the most well-known terrestrial and marine sedimentary sequences in the world. He asked the question: Is the temporal continuity of sedimentary rock over time correlated with either first or last appearances of taxa (in his case, genera), thus indicating that the two are not mechanistically independent? He showed that, in fact, almost every aspect of temporal variation in the fossil record is reflected in similar variation in the spatial and temporal distribution of sedimentary rocks. In his work, Peters (2006) also demonstrated that the total volume of terrestrial sedimentary units over North America increased dramatically over the last 90 million years. This increase in all sediments in the recent geological past is overprinted on extreme variability in the proportion of sedimentary packages constituted by fine-grained sediments (ideal for fossil plant preservation) over time. The presence of multiple marginal depositional basins is correlated with a high potential for sedimentary accumulation. An increase in sediment accumulation logically occurs when plate tectonic configurations are such that they accommodate the formation of large sedimentary basins. One implication of this result is that we should expect to find that many plant fossils appear synchronously in the fossil record, not for evolutionary reasons, but as a result of tectonic forcing of the sedimentary record. Peters' data would suggest that interpreting first occurrences as true originations is likely to be erroneous, given that the preservation potential is so much higher when tectonic plate configurations foster the accumulation of fine-grained sediments. One specific geographic location where this might have had a strong effect is in the eastern portion of Brazil, where well-preserved plant remains uniquely represent early angiosperms and gnetaleans. However, this was just the kind of tectonic setting where deposition would be predicted. So were angiosperms and gnetaleans living there preferentially, or are they simply preserved preferentially there?

Figure 5. Known Pliocene-age plant fossil localities in northern South America as of December 2006. Data for localities were extracted from the literature, with each site numbered in a database available from the author.

While this evidence might be far from the minds of those involved in phylogenetic analysis and the application of fossil plant data to constraining evolutionary scenarios, it is critical to recognize that the fossil record is both preserved and subsequently sampled in a highly nonrandom manner. Overriding considerations are the completeness of the record of any one time period, the depositional circumstances under which the original data were preserved, and the erosional circumstances of the intervening time period between deposition and exhumation.

In conclusion, this contribution addresses two important steps in fossilization and recovery that apply to a wide variety of paleobiological and evolutionary questions. Four scenarios are provided under which it can be seen that plant populations grow rapidly from a very small number of individuals to populations large enough to contribute substantively to the fossil record in a geologically short period of time (ca. 2000 years). This period of time may not be resolvable in the rock record and, as such, can be thought of as instantaneous. Migration rates of plants are generally rapid enough that there is no discernable lag time that would be produced in a fossil record if a successful plant or plant group is to be anticipated to migrate over large land areas. These data apply only to the rock record prior to the Quaternary, in which resolution is not expected on these ecological scales. Most of the modern analogies I have used to address this question are based on successful immigrants or invasives, and some are expanding under circumstances of lowered competition and predation. Therefore, the quantitative data here are recognized as representing the short end of the time-to-expansion spectrum. It is not possible to give estimates of expansion rates for a given fossil species if reproductive intervals, time to reproduction, or even ability to vegetatively reproduce are unknown for that species. Instead, it is reasonable to assume that a plant group that ultimately does contribute substantively to plant diversity, density, or vegetational importance (defined as many species within the group, biomes dominated by members of the group, continent-wide coverage by members of the group) has done well at some time in its evolutionary history, and the time scale of expansion of 10–1000 years is applicable.

In contrast, the recovery of a high-quality fossil record depends on a number of factors that are not as

easily dismissed. We should be especially cautious in assuming the adequacy of the currently known and potentially retrievable fossil record in answering detailed questions about plant evolution. While the rock record is incomplete, the analysis does not need to stop there. If the fossil record can be quantified both globally and regionally relative to the probability of bearing and preserving fossil plants, we would be able to assign confidence limits on the presences and absences that are applied from the rock record. Approaches like this have been carried out on pure sedimentary gap analysis and for paleobiodiversity analyses (see papers by Sadler & Strauss, 1989, 1990; Holland, 2000; Crampton et al., 2006) and hold great promise for the geographic and stratigraphic absences of fossil plants.

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W(H)ITHER FOSSILS? STUDYING MORPHOLOGICAL CHARACTER EVOLUTION IN THE AGE OF MOLECULAR SEQUENCES[1]

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ABSTRACT

A major challenge in the post-genomics era will be to integrate molecular sequence data from extant organisms with morphological data from fossil and extant taxa into a single, coherent picture of phylogenetic relationships; only then will these phylogenetic hypotheses be effectively applied to the study of morphological character evolution. At least two analytical approaches to solving this problem have been utilized: (1) simultaneous analysis of molecular sequence and morphological data with fossil taxa included as terminals in the analysis, and (2) the molecular scaffold approach, in which morphological data are analyzed over a molecular backbone (with constraints that force extant taxa into positions suggested by sequence data). The perceived obstacles to including fossil taxa directly in simultaneous analyses of morphological and molecular sequence data with extant taxa include: (1) that fossil taxa are missing the molecular sequence portion of the character data; (2) that morphological characters might be misleading due to convergence; and (3) character weighting, specifically how and whether to weight characters in the morphological partition relative to characters in the molecular sequence data partition. The molecular scaffold has been put forward as a potential solution to at least some of these problems. Using examples of simultaneous analyses from the literature, as well as new analyses of previously published morphological and molecular sequence data matrices for extant and fossil Chiroptera (bats), we argue that the simultaneous analysis approach is superior to the molecular scaffold approach, specifically addressing the problems to which the molecular scaffold has been suggested as a solution. Finally, the application of phylogenetic hypotheses including fossil taxa (whatever their derivation) to the study of morphological character evolution is discussed, with special emphasis on scenarios in which fossil taxa are likely to be most enlightening: (1) in determining the sequence of character evolution; (2) in determining the timing of character evolution; and (3) in making inferences about the presence or absence of characteristics in fossil taxa that may not be directly observable in the fossil record.

Key words: Character mapping, Chiroptera, convergence, echolocation, fossil, homoplasy, molecular scaffold, molecular sequence data, morphological character evolution, phylogeny, simultaneous analysis, total evidence.

At one time, extinct taxa represented by fossils (hereafter, fossil taxa) were considered central to understanding the evolution of organisms through time (see, for instance, Eldredge & Cracraft, 1980; Smith, 1998). Phylogenetic hypotheses were developed by a qualified expert or experts on the basis of comparative anatomy and morphology, to which fossils were considered to contribute primitive and intermediate forms through which one could trace evolution from ancestor to descendant to the most recent members of a group. Characters considered meaningful to the development of evolutionary scenarios were entirely at the discretion of the investigator, and overall similarity as well as the appearance of advanced features (i.e., synapomorphies) were considered important in interpreting relationships. With the advent of the framework explicated by Hennig (1966) and the development of analytical methodologies and programs for tree-building (e.g., Farris, 1970; Fitch, 1971; Felsenstein, 1981), paleontology became less central to understanding evolution through geologic time (despite the early recognition of the logic and utility of the cladistic methodology by some paleontologists [e.g., Schaeffer et al., 1972]) because extant organisms could be grouped on the basis of shared derived traits—or synapomorphies—without reference to the fossil record. In fact, fossil taxa, for which many data were often missing and whose interpreta-

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tion was potentially difficult, became viewed by some as an impediment to understanding phylogenetic relationships among extant taxa (e.g., Patterson, 1981). Molecular systematics, which provides large numbers of sequence characters, has altered our understanding of the relationships among and within many groups, often without reference to fossil taxa.

However, fossil taxa provide unique types of information not available in extant organisms, and, because of this, the recognition of fossil taxa as an important component of phylogenetic studies has recently experienced a renaissance (Smith, 1998). Some of this may be due to the temporal information that fossil taxa can provide about the rate and timing of group diversification, principally in the application of temporal data associated with the occurrences of fossils as calibration points in studies of the rate of evolution of molecular sequence characters (e.g., Peterson et al., 2004; Schneider et al., 2004). Fossils are also unique repositories of data on extinct morphologies for groups both with and without representation in the extant biota. Thus, fossil taxa can provide insight into the sequence of evolution within morphological characters that are correlated in extant taxa, as well as access to suites of characters or variations within characters that would be entirely lost if not for knowledge of the vast extinct flora and fauna that once flourished on earth (Donoghue et al., 1989; Smith, 1998; Forey & Fortey, 2001).

One of the biggest challenges for paleontologists and systematists alike in the post-genomics era will be to figure out how best to incorporate paleontological data (primarily anatomical and morphological data, hereafter simply referred to as morphological data) with molecular sequence data from extant organisms to take advantage of these unique aspects of fossil taxa (e.g., see Peterson et al., 2007). The issues involved in this subject are complex, ranging from character delimitation and interpretation, the effect of missing data on analyses, and whether to combine data sets and analyze fossil taxa directly with extant taxa—referred to as simultaneous analysis (Nixon & Carpenter, 1996) or combined analysis, or the total evidence (Kluge, 1989) or supermatrix approach (see review in de Queiroz & Gatesy, 2007)—or to use more indirect methods, such as trees based on molecular backbone constraints, sometimes referred to as "molecular scaffolds" (Springer et al., 2001: 6242). Herein, we compare several methods for combining morphological and molecular sequence data for a group (Chiroptera, bats) that has attributes (e.g., a large body of molecular sequence data conflicting with traditional groupings based on morphology, several well-preserved fossil representatives) emblematic of the current problems confronting the integration of extant with fossil taxa in which molecular sequence data are involved. We will use these analyses as examples in a review of major issues surrounding tree-building and the interpretation of character evolution in joint fossil-extant taxon analyses that include a molecular sequence and morphological component. In the first part of this discussion, we argue that, because many of the issues surrounding character mapping on a phylogeny are not unique to analyses in which fossil taxa are included, the underlying problem in studying fossil taxa in a phylogenetic context is to identify the most effective way to integrate our knowledge of morphological characters with our evolving knowledge of the tree of relationships among extant organisms as suggested by molecular sequence data. In the second part, we discuss the utility and complications of mapping characters and studying character evolution in a context in which fossil taxa are included as terminals in a phylogenetic analysis, emphasizing examples from simultaneous analyses.

Background

The Cenozoic record of the placental mammal clade Chiroptera (bats) provides a good data set to explore how paleontological, morphological, and molecular sequence data interact in phylogenetic analyses, and how, in turn, these data types can inform hypotheses of the sequence and timing of morphological character evolution. A plethora of morphological and molecular sequence data has been collected about bats, and several analyses have integrated data sets in order to explore patterns of character evolution and biogeography within both extant and fossil members of the Chiroptera (Springer et al., 2001; Teeling et al., 2005). Traditionally, bats have been grouped by morphological data into two clades, Microchiroptera (microbats), with laryngeal echolocation (a biological form of sonar to hunt prey), and Megachiroptera (Pteropodidae; flying foxes and Old World fruit bats), lacking laryngeal echolocation (Simmons, 2005a). Recently, analyses of molecular sequence data have challenged this traditional view of bat evolution (see reviews in Simmons, 2005a; Jones & Teeling, 2006); these data suggest that Pteropodidae (flying foxes and Old World fruit bats) and an echolocating microbat group called Rhinolophoidea (horseshoe bats) are more closely related to one another than either is to the remaining microbats. The clade including Pteropodidae and Rhinolophoidea is known as Yinpterochiroptera, while the clade including other echolocating bats has been referred to as Yangochiroptera (Springer et al., 2001). While it was once thought that laryngeal echolocation—which has a complex morphological basis (Arita

& Fenton, 1997)—evolved only once in bats, the new view of bat phylogeny based on molecular sequence data raises the possibility that laryngeal echolocation either evolved twice independently, or evolved once, but was lost in Pteropodidae (Springer et al., 2001; Jones & Teeling, 2006).

To date, direct simultaneous analyses (Nixon & Carpenter, 1996) of combined morphological and molecular sequence data sets from extant families across the order Chiroptera (with or without fossil taxa) are lacking, despite the potential that these combined data may have for further clarifying the relationships of bats (Simmons, 2005a) and the evolution of echolocation. Here, we explore whether performing such an analysis will support the new molecular view of bat phylogeny, as predicted by Simmons (2005a: 167). Prior research in this area has been undertaken only indirectly: Springer et al. (2001) and Teeling et al. (2005) used a molecular scaffold approach (e.g., used backbone constraints) to place fossil taxa within a phylogenetic context.

Materials and Methods

Combined Data Matrix

The combined morphological and molecular sequence data set analyzed here was constructed from two previously published data matrices. The NEXUS data file representing the morphological matrix published by Gunnell and Simmons (2005) was downloaded from the American Museum of Natural History FTP site linked directly from Nancy Simmons' homepage (http://research.amnh.org/mammalogy/personnel/simmons.php). This morphological matrix features a total of 35 terminal taxa, six of which are fossil taxa—*Archaeonycteris* Revilliod, *Hassianycteris* Smith & Storch, *Icaronycteris* Jepsen, *Palaeochiropteryx* Revilliod, *Tanzanycteris* Gunnell et al., and an undescribed genus from the Eocene Green River Formation of Wyoming (Gunnell & Simmons, 2005)—and five of which are extant outgroup taxa (*Cynocephalus* Boddaert, flying lemur; *Erinaceus* L., hedgehog; *Felis* L., cat; *Sus* L., pig; and *Tupaia* Raffles, tree shrew). There are 204 morphological characters, 94 of which are soft tissue and 110 of which are osteological characters; 165 have nonadditive (unordered) transformations and 39 have additive (ordered) transformations. All extant ingroup terminals (n = 24) in this morphological matrix are extant familial or subfamilial bat taxa. Fossil taxa are scored at the genus level.

The aligned molecular sequence data matrix, the basis for the study by Teeling et al. (2005), was kindly provided to the authors (specifically, JRH) by Dr. Teeling on June 9, 2006. The complete molecular sequence data matrix includes 13,792 aligned sequence characters representing "nuclear sequence data from portions of 17 nuclear genes" (Teeling et al., 2005: 581) from 30 bat genera (representing all families of Chiroptera; sequence data for some ingroup terminal genera are composites from multiple infrageneric species) and four outgroup terminals represented by composite sequence data gathered from two or more genera each. Details of this matrix, including GenBank accession numbers, were provided by Teeling et al. (2005; supplementary table S6).

Three options for reconciling overlapping taxa between the two matrices presented themselves at the beginning of this study: (1) culling taxa from the molecular sequence data set, leaving one generic-level terminal to combine with the corresponding family or subfamily terminal in the morphological data set; (2) fusing terminals in the molecular sequence data set so that all molecular sequence variability for each family or subfamily was encompassed in one corresponding terminal in the morphological matrix; or (3) duplicating morphological terminals in order that each terminal represented by a molecular sequence data set also had a morphological data set, some of which would be identical for members of the same family or subfamily. Each option has potential pitfalls. The first would discard the most data, the second would result in an increase of polymorphisms in the molecular sequence data set, and the third would result in multiple terminals sharing the same set of morphological characters. For a more generalized discussion of the problem of terminal mismatch in combining data matrices, see Nixon and Carpenter (1996).

We decided to use option three (duplication of morphological terminals), because at least some studies have suggested that phylogenetic accuracy increases with greater taxon sampling (e.g., Zwickl & Hillis, 2002), and we did not wish to discard information; further, we did not want to add to the ambiguity of the combined data set (which already includes many cells coded as missing) by fusing molecular sequence terminals. This option allowed us to keep all terminals represented in the molecular sequence data set except Perissodactyla (odd-toed hoofed mammals), for which we could find no reasonable combination with a terminal in the morphological data set (see further discussion below). The number of terminals represented by molecular sequence data that share the same duplicated morphological data in the combined data set range from zero (17 taxa, including three of the outgroups) to two (two groups of two taxa), three (one group of three taxa), or four (two groups of four taxa). For genera with identical morphological data sets, the morphological data obviously supply no information on intrafamilial

relationships; in the simultaneous and molecular scaffold analyses, these are completely structured by the molecular sequence data.

One potentially problematic aspect of the morphological data set is that, where polymorphisms existed in the earlier Simmons and Geisler (1998) morphological matrix, of which the Gunnell and Simmons (2005) matrix is a modification, Gunnell and Simmons (2005) replaced them either with an inferred ancestral state (IAS) for the family or subfamily (IAS coding) or with the most common state in the family or subfamily, or used ambiguity coding for that character (see Simmons & Geisler, 2002). Simmons and Geisler (1998) listed the number and percent of polymorphisms within each terminal in the previous version of this matrix, thus giving some indication of how many cells may have been converted from polymorphic to single state for each terminal in Gunnell and Simmons (2005). This means that, in cases in which polymorphisms may occur intrafamilially or intrasubfamilially, the inferred plesiomorphic state within the higher-level terminal may be substituted for a polymorphism (Simmons & Geisler, 2002), and this state may or may not occur in the genus matched with the higher-level terminal. Hence, the analyses could be improved in the future by coding the states present in individual genera, rather than in families or subfamilies. As a corollary, not all characters coded for each higher-level terminal may have been observed in each genus included in the molecular sequence matrix, so some extrapolation of character states within genera may be occurring (see Nixon & Carpenter [1996] concerning extrapolation). According to Simmons (2005c: 527), extant bats are classified into 18 families, another six families are known from fossils, and "biologists have long agreed that these groups represent distinct evolutionary lineages," although "there has been no consensus concerning relationships among them." Because the monophyly of the families within Chiroptera is apparently not in question and is further supported by the molecular sequence data set employed here (Teeling et al., 2005), we do not anticipate error caused by incorrectly assigning some genera to the wrong family. According to Simmons and Geisler (1998), monophyly of all bat taxa (families and subfamilies) included as terminals in the analysis is well established, except perhaps for Vespertilioninae; only one genus, *Rhogeessa* H. Allen, is assigned to Vespertilioninae in this study.

Combining these two matrices required renaming the terminal extant bat taxa in the morphological matrix (suprageneric) with the generic terminal names in the molecular sequence data matrix. The classification of Simmons (2005b) was used to match each extant bat genus with the suprageneric taxon to which it belongs (see Table 1). For the outgroups, the composite terminal *Felis/Panthera* Oken in the molecular sequence data set was matched to the morphological data set for *Felis* and named *Felis*; the composite terminal *Condylura* Illiger/*Talpa* L./*Scalopus* Desmarest (moles) was matched with *Erinaceus* (hedgehog), and these were renamed Eulipotyphla, a monophyletic clade composed of some former members of the Insectivora, including hedgehogs, shrews, and moles (e.g., Murphy et al., 2001). Finally, the composite molecular sequence terminal *Tragelaphus* Blainville/*Bos* L. (bovines) were combined with the morphological data for *Sus* (pigs) to form the terminal Cetartiodactyla, a clade supported by molecular sequence data (e.g., Montgelard et al., 1997; Murphy et al., 2001; Boisserie et al., 2005) that also includes additional ruminants, whales, and hippopotami. The Perissodactyla outgroup, composed of sequence data from *Equus* L. (horses) and *Ceratotherium* Gray (rhinoceroses), could not be combined with a morphological terminal, as no perissodactyls are outgroups in the morphological matrix. Because it lacks morphological data, the Perissodactyla outgroup can then act as a "wild card" (Nixon & Wheeler, 1992: 134; see discussion below), interacting with fossil bat taxa that group between the outgroup taxa and extant ingroup bats, since Perissodactyla and the fossil bat taxa are coded for mutually exclusive data sets. Perissodactyla was thus removed from the combined matrix. Four extant bat subfamilies and two outgroup terminals represented in the Gunnell and Simmons (2005) matrix that are not represented at all in the Teeling et al. (2005) data set were allowed to remain in the combined matrix, since they were all coded for the morphological characters. These taxa were thus similar to (but more complete than) the fossil taxa. The total combined morphological and molecular sequence data set (hereafter referred to as the combined data set) included 45 terminal taxa (five outgroups) and 13,996 characters (39 additive). All characters were weighted equally.

CLADISTIC ANALYSES

Terminals were duplicated and renamed in WordPad (Microsoft Corporation, Redmond, Washington), matrix dimensions were modified (where necessary), and the files were opened in WinClada (Nixon, 1999–2002). Matrices were combined in WinClada, with terminals matched as detailed in Table 1. The combined data set was saved in .ss format before it was opened in the software program TNT (Goloboff et al., 2003a), where it was resaved in TNT format. Tree searches were performed under the parsimony criterion using TNT (Goloboff et al., 2003a). For each

Table 1. Morphological (family and subfamily) data set matched to each molecular sequence (genus) terminal. Assignments of genera to higher taxa follow Simmons (2005b). Abbreviations in the molecular sequence data column correspond to the GenBank molecular sequence accession information in supplementary table S6 of Teeling et al. (2005); the first letter corresponds to the genus name, the next two letters to the molecular source species name, and the number in the bracket refers to composite terminal numbers in table S6 of Teeling et al. (2005). Four extant terminal taxa possess morphological data (from Gunnell & Simmons, 2005) but lack corresponding molecular sequence data; these include Tomopeatinae Miller (Molossidae) and Miniopterinae Dobson, Murininae Miller, and Kerivoulinae Miller (Vespertilionidae).

Extant bat genus	Taxonomic level (family or subfamily) from which morphological data were coded[1]	Molecular sequence data source[2]
Antrozous H. Allen	Antrozoidae	Apa[19]
Craseonycteris Hill	Craseonycteridae	Cth[30]
Emballonura Temminck	Emballonuridae	Eat[11]
Taphozous E. Geoffroy	Emballonuridae	Tnu[12]
Rhynchonycteris Peters	Emballonuridae	Rna[13]
Furipterus Bonaparte	Furipteridae	Fho[26]
Hipposideros Gray	Hipposideridae	Hco[6]
Megaderma E. Geoffroy	Megadermatidae	Mly[7]
Macroderma Miller	Megadermatidae	Mgi[8]
Tadarida Rafinesque	Molossinae	Tbr[28]
Eumops Miller	Molossinae	Eau[29]
Pteronotus Gray	Mormoopidae	Ppa[23]
Myotis Kaup	Myotinae	Mda[21]
Mystacina Gray	Mystacinidae	Mtu[25]
Myzopoda Milne-Edwards & A. Grandidier	Myzopodidae	Mau[22]
Natalus Gray	Natalidae	Nst[27]
Noctilio L.	Noctilionidae	Noctal[18]
Nycteris G. Cuvier & E. Geoffroy	Nycteridae	Ngr[9]
Tonatia Gray	Phyllostomidae	Tsi[14]
Artibeus Leach	Phyllostomidae	Aja[15]
Desmodus Wied-Neuwied	Phyllostomidae	Dro[16]
Inoura Gray	Phyllostomidae	Age[17]
Pteropus Erxleben	Pteropodidae	Pgi[1]
Cynopterus F. Cuvier	Pteropodidae	Cbr[2]
Rousettus Gray	Pteropodidae	Rla[3]
Nyctimene Borkhausen	Pteropodidae	Nal[4]
Rhinolophus Lacépède	Rhinolophidae	Rcr[5]
Rhinopoma E. Geoffroy	Rhinopomatidae	Rha[10]
Thyroptera Spix	Thyropteridae	Ttr[24]
Rhogeessa H. Allen	Vespertilioninae	Rtu[20]

[1] From Gunnell and Simmons, 2005.
[2] From Teeling et al., 2005.

analysis, the collapsing rule (determining which nodes will be collapsed from dichotomous to polytomous in most parsimonious trees [MPTs]) was set to rule 3, which only collapses nodes with no character support (max length = 0). For each analysis, a heuristic search was employed using the following parameters: starting Wagner trees were calculated using a random seed of 0; 1000 search replications were performed with tree bisection-reconnection branch-swapping (Swofford & Olsen, 1990), saving up to 10 shortest trees per search replication. Trees were collapsed after the search (in other words, branches with a maximum length of 0 were collapsed into polytomies rather than being displayed as dichotomies). The ensemble consistency index (CI; Kluge & Farris, 1969) and ensemble retention index (RI; Farris, 1989) were calculated based on the total minimum and maximum number of steps for each matrix as calculated by WinClada.

For analysis 1a, the modified morphological data partition was analyzed without constraints. This analysis was performed to confirm the results of the original Gunnell and Simmons (2005) analysis with a matrix including the cloned terminals. For analysis 1b, bat genera represented in the molecular sequence data matrix (Table 1) were constrained to conform

with the Yinpterochiroptera–Yangochiroptera groupings as shown in Teeling et al. (2005). Only two positive constraints (one for each clade) were used. Fossil taxa and supergeneric extant ingroup taxa were designated as floaters (unconstrained). This analysis was performed to determine how many additional steps would have to occur in the morphological data in order for them to support the major dichotomy in the Chiroptera suggested by the molecular sequence data set (Teeling et al., 2005).

For analysis 2a, the modified Teeling et al. (2005) matrix was analyzed without constraints. For analysis 2b, the outgroup Perissodactyla was deactivated and the modified Teeling et al. (2005) data set was analyzed. The purpose of this analysis was to insure that the Yinpterochiroptera and Yangochiroptera were still recovered as monophyletic clades with Perissodactyla removed from the molecular sequence data set. For analysis 2c, the molecular sequence data matrix minus Perissodactyla was analyzed with taxa traditionally assigned to Megachiroptera and Microchiroptera constrained to belong to those groups (two positive constraints). The purpose of this analysis was to determine how many additional steps must occur in the molecular sequence data in order for them to support the major dichotomy in the Chiroptera suggested by the morphological data (Gunnell & Simmons, 2005) and traditional classifications.

For analysis 3a, the combined data matrix was analyzed with only those taxa for which both data partitions were coded (other taxa were deactivated). The purpose of this analysis was to determine whether taxa with large amounts of missing data were significantly affecting the results of the simultaneous analysis with fossil and extant taxa. For analysis 3b, the combined data set with all taxa except Perissodactyla was analyzed. For analysis 3c, the combined data set was analyzed with Yinpterochirpotera–Yangochirptera constraints, as in analysis 1b.

For analysis 4, all nodes from the molecular tree topology found in analysis 2b were constrained, and only taxa lacking molecular sequence data were allowed to float. This analysis emulates the methods of Teeling et al. (2005) and others (see below).

CHARACTER MAPPING

The binary (presence/absence) laryngeal echolocation character was mapped onto the trees resulting from analysis 3b using Fitch optimization (Fitch, 1971).

RESULTS

Table 2 summarizes the results for each analysis, including number of MPTs, length of MPTs, and CI and RI. Topological results are discussed below as relevant (e.g., when analyses were not performed under constraints). For analysis 1a (morphological data with no constraints), the strict consensus of all MPTs was concordant with the results shown in Gunnell and Simmons (2005: fig. 1). The strict consensus of the two MPTs for analysis 2a (the molecular sequence data set without constraints) has the same overall structure as that shown in Teeling et al. (2005: fig. 1), which illustrated the maximum likelihood (ML) tree calculated under the GTR + Γ + I model of molecular sequence evolution. In both trees, Yinpterochiroptera and Yangochiroptera are monophyletic sister groups composed of the same taxa. The Pteropodidae (flying foxes and Old World fruit bats) and Rhinolophoidea (horseshoe bats) form monophyletic clades sister to one another in Yinpterochiroptera, although the internal arrangement of Pteropodidae is different in our parsimony and the ML trees of Teeling et al. (2005). Emballonuridae, Phyllostomidae, Vespertilionidae, and Molossidae form monophyletic groups within Yangochiroptera (other families are represented by only one terminal), although the internal structure of Yangochiroptera is both different from and less resolved in the strict consensus of the parsimony trees found here as compared to the ML tree (see Teeling et al., 2005: fig. 1). The results of analysis 2b (the molecular sequence data set with no constraints, Perissodactyla deactivated) also support the Yinpterochiroptera–Yangochiroptera groupings. Support values—standard bootstrap (Felsenstein, 1985), Poisson bootstrap (Farris et al. in Horovitz, 1999b), and symmetrical resampling (Goloboff et al., 2003b) with traditional search—calculated for this pruned data set suggest that removing Perissodactyla does decrease the support (as expressed as absolute frequency) for the Yinpterochiroptera clade, although the degree to which support was affected was dependent on the resampling procedure used. Support values for Yinpterochiroptera ranged from 75 (5000 replicates of standard bootstrapping) to 89 (5000 replicates of symmetrical resampling at 33% change probability), as compared to 96 as reported by Teeling et al. (2005) when Perissodactyla was included (1000 replicates of standard bootstrapping in PAUP 4.10b10 [Swofford, 2003]).

The results of analysis 3a (the unconstrained combined data set including taxa with both data partitions only) support the traditional groupings (monophyletic Microchiroptera and monophyletic Megachiroptera) among extant bats. Similarly, the results of analysis 3b (the combined data set without constraints, Perissodactyla deactivated) do not support paraphyly of Microchiroptera with respect to Megachiroptera. Two fossil bat taxa, *Icaronycteris* and the

Table 2. Results of phylogenetic analyses. Descriptions of analyses are as follows (see text for further details): analysis 1a, morphological data set; 1b, morphological data set with Yinpterochiroptera–Yangochiroptera constraints; 2a, molecular sequence data set, all outgroups; 2b, molecular sequence data set, Perissodactyla deactivated; 2c, molecular sequence data set with Megachiroptera–Microchiroptera constraints, Perissodactyla deactivated; 3a, combined data set, extant taxa only; 3b, combined data set, extant and fossil taxa; 3c, combined data set, Yinpterochiroptera–Yangochiroptera constraints; and 4, molecular scaffold analysis, all nodes constrained. MPTs = most parsimonious trees.

	Analysis								
	1a	1b	2a	2b	2c	3a	3b	3c	4
Taxa (outgroup, ingroup, fossil)	45 (5, 40, 6)	45 (5, 40, 6)	34 (4, 30, 0)	33 (3, 30, 0)	33 (3, 30, 0)	39 (5, 34, 0)	45 (5, 40, 6)	45 (5, 40, 6)	45 (5, 40, 6)
Characters (total and no. informative)	204 (202)	204 (202)	13792 (3792)	13792 (3533)	13792 (3533)	13996 (3735)	13996 (3735)	13996 (3735)	NA
MPTs	4	30	2	1	2	3	16	1	3
Morphological characters									
Length	791	827	NA	NA	NA	750, 763, 771	875 (8), 888 (8)	901	923
CI	0.363	0.347	NA	NA	NA	0.357, 0.360, 0.367	0.323 (8), 0.328 (8)	0.319	0.311
RI	0.732	0.713	NA	NA	NA	0.659, 0.664, 0.673	0.681 (8), 0.688 (8)	0.674	0.662
Molecular characters									
Length	NA	NA	15782	15238	15249	15258, 15271, 15250	15258 (8), 15271 (8)	15248	15238
CI	NA	NA	0.531	0.539	0.538	0.537, 0.538 (2)	0.537 (8), 0.538 (8)	0.538	0.539
RI	NA	NA	0.485	0.491	0.490	0.489 (2), 0.490	0.488 (8), 0.489 (8)	0.490	0.491
All characters									
Length	NA	NA	NA	NA	NA	16021	16146	16149	16161
CI	NA	NA	NA	NA	NA	0.529	0.526	0.526	0.526
RI	NA	NA	NA	NA	NA	0.506	0.512	0.512	0.511

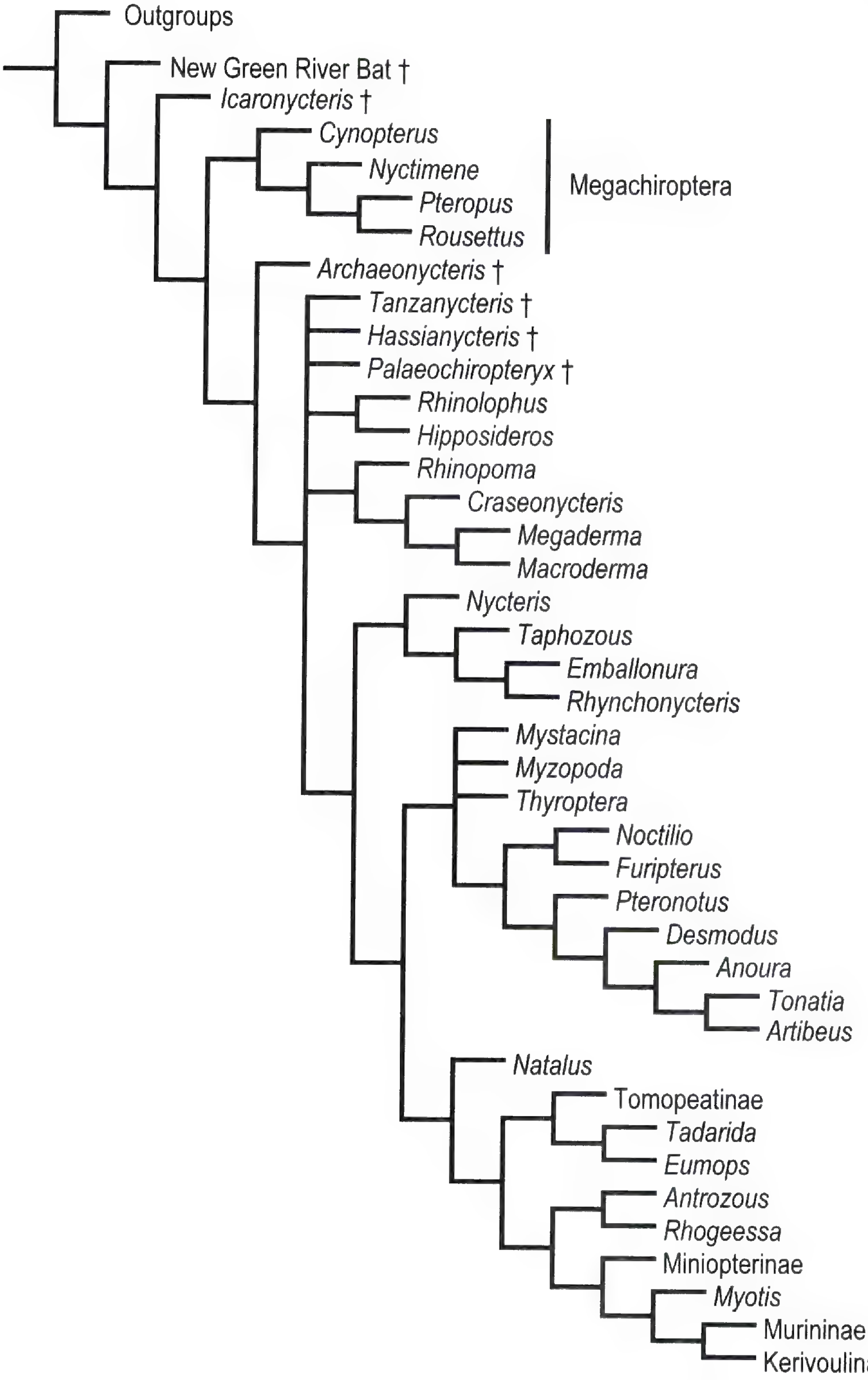

Figure 1. Strict consensus of 16 MPTs (16,146 steps; CI = 0.526; RI = 0.512) resulting from the analysis (3b) of the combined morphological and molecular sequence data set, including all extant and fossil taxa, without constraints. Fossil taxa are indicated by a dagger (†).

Green River Bat, group outside of all extant bats. The other four fossil bat taxa and extant Microchiroptera form a monophyletic group (Fig. 1). The clade that includes Rhinolophidae, Megadermatidae, Craseonycteridae, and Rhinopomatidae is partially collapsed due in part to interaction with the fossil taxa (Fig. 1).

The strict consensus of three MPTs found during analysis 4 (the molecular scaffold with all nodes constrained, Perissodactyla deactivated) is shown in Figure 2. *Tanzanycteris* is resolved sister to Rhinolophidae. In one tree, *Hassianycteris* is sister to all Yangochiroptera and in the other trees is outside of the clade including all extant bats. All other fossil taxa are on the stem lineage of extant bats in all MPTs.

Discussion

The most basic problem of studying character evolution in a phylogenetic context is a problem of methodology: how will the trees be built? Different methodologies have been employed to integrate data from fossil taxa (primarily composed of morphological data) and extant taxa (now including, or often composed entirely of molecular sequence data) in analyses that incorporate information from multiple sources. One of these is simply to suggest the position of a fossil taxon on a tree of extant taxa by reference to morphological synapomorphies mapped on a tree (e.g., Rowe, 1988, for select fossil taxa with more than 12% missing data; Boucher et al., 2003). Two others, which take a more analytical approach, are the molecular scaffold (also known as molecular backbone constraints, molecular constraints, etc.) and simultaneous analysis (also known as total evidence or combined analysis, or the supermatrix approach). The first of these, the molecular scaffold, gives greater precedence (at least to some degree, depending on the constraints used) to the topology suggested by the molecular sequence data. In this type of analysis, extant taxa are analyzed using molecular sequence data, all or some of the relationships among these taxa on the resultant tree(s) are constrained, and then a morphological matrix including fossil taxa is analyzed under the constraints (e.g., Springer et al., 2001; Sánchez-Villagra et al., 2003; Roca et al., 2004: supplementary data; Asher et al., 2005a; Teeling et al., 2005). In a total evidence approach (Kluge, 1989) or a combined or simultaneous analysis (Nixon & Carpenter, 1996), all character data are combined into a single supermatrix (see de Queiroz & Gatesy, 2007), and extant and fossil taxa are analyzed together. In the latter approach, morphological data can have a greater influence on the resultant tree topologies, and inclusion of morphological data with molecular sequence data has sometimes been shown to significantly alter the topologies recovered relative to those found when sequence data are analyzed alone (see discussion below). Generally, simultaneous analyses have been performed under equal-weights parsimony with sequence data aligned prior to analysis, although some authors (e.g., Giribet et al., 2002; Asher et al., 2003, 2004; Wheeler et al., 2004; Arango & Wheeler, 2007) have chosen to implement direct optimization of sequence data (Wheeler, 1996, 2003) during phylogeny reconstruction, in which different costs can be assigned to morphological and various types of molecular transformations.

Inclusion of fossil taxa in phylogenetic analyses increases taxon sampling and does so in a very unique way. Fossil taxa represent lineages sampled through time and, as such, can be repositories of unique morphologies that may not be represented in today's biota. Thus, direct inclusion of fossil taxa in phylogenetic analyses, rather than overlaying a morphological analysis onto a molecular scaffold, can alter tree topologies, sometimes in ways that yield different results than simply combining data partitions for extant taxa alone. In fact, the addition of fossil taxa representing extinct diversity has the potential to alter the interpretation of relationships among extant taxa any time homoplasy occurs in the data set on which a phylogenetic hypothesis is based (Nixon & Wheeler, 1992). Thus, effectively, fossil taxa that possess unique combinations of characters almost always have the potential to alter the hypothesis of phylogenetic relationships when included in an analysis and, in certain situations (e.g., cases in which large accumulations of apomorphies distinguish extant taxa; see Donoghue et al., 1989), might be expected to significantly affect the perceived relationships among extant taxa.

Perhaps the earliest illustration of this property in a combined analysis of morphological and molecular sequence data was presented by Eernisse and Kluge (1993), who studied amniote relationships. They performed analyses including morphological (Gauthier et al., 1988) and molecular sequence (Hedges et al., 1990) data sets in various combinations with and without fossil taxa. In some pairs of analyses (e.g., combined 18S rRNA sequences plus morphology), inclusion of fossil taxa was critical; without fossil taxa, birds and mammals formed a monophyletic group to the exclusion of crocodiles, turtles, and lepidosaurs (e.g., snakes, lizards, tuataras); with fossil taxa, birds and crocodiles were sister taxa in a monophyletic group with turtles and lepidosaurs to the exclusion of mammals. When all characters were considered, the results were consistent with a monophyletic bird-extant reptile clade, although the positions of turtles

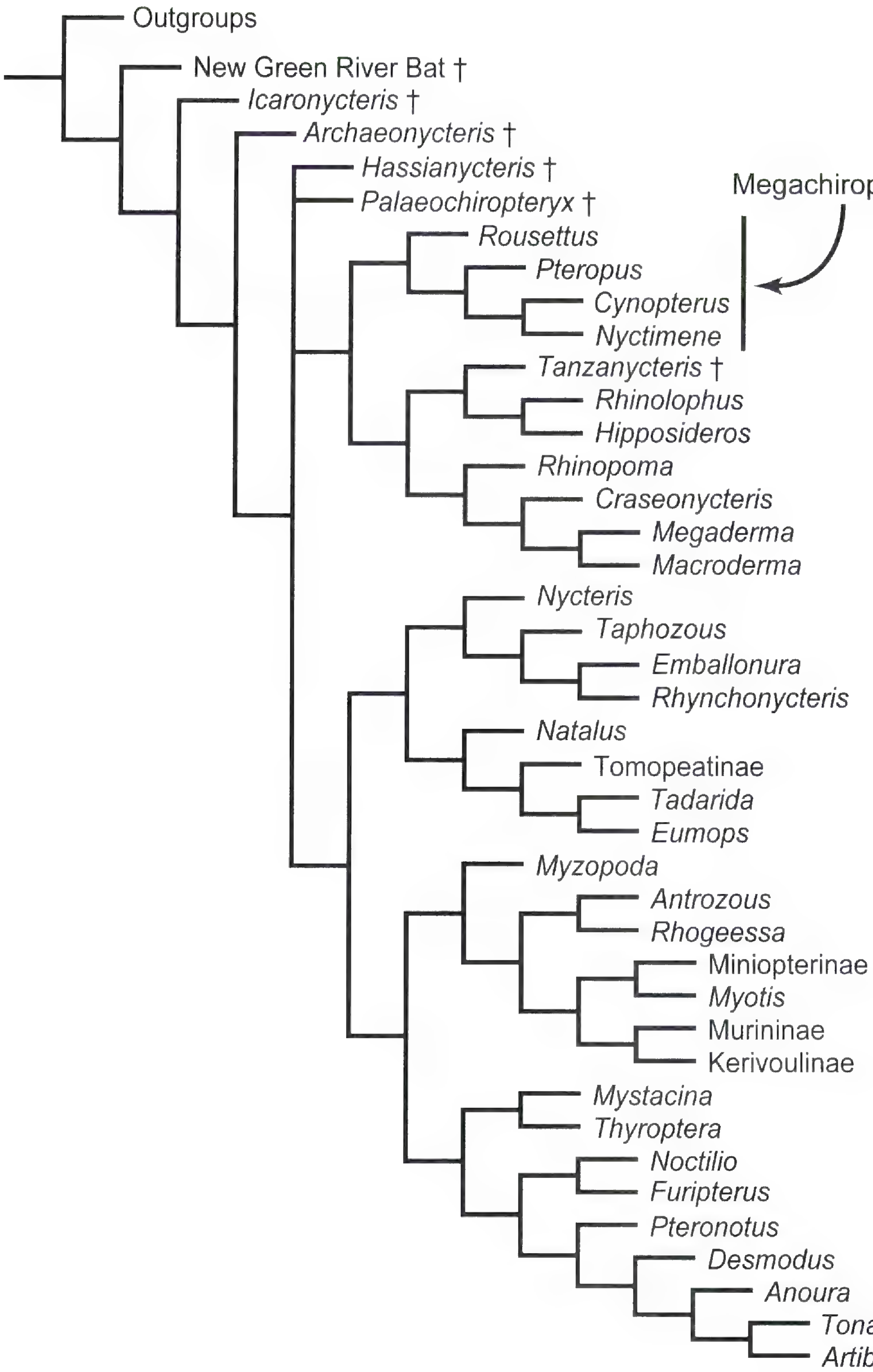

Figure 2. Strict consensus of three MPTs resulting from analysis (4) of morphological data over a molecular scaffold under full constraints (all taxa with molecular sequence data constrained). Taxa lacking the molecular sequence data partition were analyzed without constraints. Tree statistics are given in Table 2. Fossil taxa are indicated by a dagger (†).

and lepidosaurs were reversed among extant-only and fossil-extant analyses. Another demonstration of the difference that inclusion of fossil taxa can make was provided by Wheeler et al. (2004), which showed that including fossil taxa in a supermatrix of morphological and molecular sequence data for arthropods resulted in some differences in relationships among extant groups relative to an analysis with extant taxa alone. For example, the extant-only analyses always resolved Crustacea (e.g., crabs, shrimps, and barnacles) and Hexapoda (insects) as sister groups, whereas the fossil-extant analyses sometimes instead resolved Hexapoda and Myriapoda (e.g., centipedes and millipedes) as sister groups, depending on the cost ratios used to optimize the molecular data. A recent study of euphyllophytes by Rothwell and Nixon (2006) compared simultaneous analyses of sequence data (Pryer et al., 2001) with morphological data (Pryer et al., 2001, with addition of fossil taxa by Rothwell & Nixon, 2006) when fossil taxa were included and excluded. One of the notable differences in the resultant parsimony topologies was that lycophytes were a stem taxon of the euphyllophytes in the extant-only analyses (Pryer et al., 2001), whereas they were sister to the lignophytes when fossil taxa were included and the extant outgroups (bryophytes) were replaced with a fossil outgroup taxon presumably more closely related to the lignophytes (Rothwell & Nixon, 2006).

Few studies to date have directly compared the results of fossil-extant molecular scaffold and simultaneous analyses, specifically considering the effect each approach may have on the relative placements of fossil and extant taxa. Asher et al. (2005a) compared simultaneous and molecular scaffold analyses of fossil and extant placental mammals with the goal of exploring the affinities of the fossil lipotyphlan (mammalian insectivores) genus *Centetodon* Marsh. Simultaneous analyses indicated that *Centetodon* grouped with Eulipotyphla (insectivores) in a more derived position than *Solenodon* Brandt (solenodons, insectivorous mammals endemic to the Caribbean). The molecular scaffold analysis also indicated that *Centetodon* belongs within Eulioptypha but did not decisively resolve the position of *Centetodon* relative to *Solenodon*. There were many substantive differences in the inferred interrelationships of mammalian orders between the supermatrix and molecular scaffold topologies. Manos et al. (2007) compared simultaneous and molecular scaffold analyses of fossil and extant members of the angiosperm family Juglandaceae (walnut family). Both analyses recovered two clades (englehardoids and juglandoids) within the family, and the strict consensus trees resulting from each analysis were similar. The positions of the fossil taxa were also similar between analyses, the biggest difference being that *Paleooreomunnea stoneana* Dilcher, Potter & Crepet (a fruit taxon; Dilcher et al., 1976) grouped with the juglandoids in the simultaneous but not the molecular scaffold analysis. Magallón (2007) compared simultaneous and molecular scaffold analyses of fossil and extant taxa in the angiosperm family Hamamelidaceae (witch hazel family). The strict consensus of the simultaneous analysis was poorly resolved, whereas the strict consensus of the molecular scaffold analysis had greater resolution. In both analyses, the fossil taxon *Archamamelis* Endress & Friis (a floral taxon; Endress & Friis, 1991) was resolved sister to *Hamamelis* L. (witch hazel), although the relationships of the other fossil taxa were more ambiguous. The results of our study show a clear difference in the positions of fossil taxa between simultaneous and molecular scaffold analyses, with fossil taxa being divided into two stem group and four crown group taxa in the unconstrained combined analysis (3b, Fig. 1) and five stem group taxa and one crown group taxon or four stem group and two crown group taxa in the molecular scaffold analysis (4, Fig. 2). The difference in the basal dichotomy among extant bats between the two analyses—caused by addition of morphological data to the pruned molecular sequence data set in the simultaneous analysis, as demonstrated when the combined data set is analyzed with all taxa lacking the molecular partition removed (analysis 3a)—is likely affecting the inferred positions of the fossil taxa.

Comparison of the results of simultaneous and molecular scaffold analyses and simultaneous analyses with and without fossil taxa clearly demonstrates, even though examples are relatively few, that choice of methodology can affect the optimal topologies and, thus, that the type of analysis performed and/or the direct inclusion of fossil taxa does matter. The rationale for using a total evidence or simultaneous analysis approach to analyzing data (not necessarily including fossil taxa) was made by Kluge (1989), later by Nixon and Carpenter (1996), and more recently by de Queiroz and Gatesy (2007); perhaps the most persuasive argument for such an approach is that all putatively phylogenetically informative data should be used to construct phylogenetic hypotheses. Recent arguments against simultaneous analysis of morphological with molecular sequence data, against use of select morphological characters (those that are incongruent with a molecular scaffold), and/or for the molecular scaffold approach, include: (1) that the most generally accepted technique for analyzing morphological data is parsimony, whereas molecular sequence data may be better analyzed using other methods (e.g., Springer et al., 2001; Asher et al.,

2005a); (2) that molecular sequence data are not available for most fossil taxa, or the missing data argument (e.g., Springer et al., 2001; Manos et al., 2007); (3) that morphological data are subject to homoplasy due to convergence (e.g., Springer et al., 2004, 2007; Eick et al., 2005); and (4) that simultaneous analyses "fail to address the weighting problem posed by including molecular and morphological data in the same data matrix" (Springer et al., 2004: 436). The first point is certainly debatable with regard to molecular sequence data sets (e.g., Frost et al., 2001) and is becoming moot with regard to morphological data (Lewis, 2001; also noted by Springer et al., 2004). For example, recent analyses of morphological (e.g., Müller & Reisz, 2006) or combined morphological and molecular sequence data (Glenner et al., 2004; Nylander et al., 2004), sometimes including fossil taxa (Lee, 2005; Xiang et al., 2005; Asher & Hofreiter, 2006; Müller & Reisz, 2006), have used Bayesian methods, although this approach is relatively little explored for both phylogeny building and the study of character evolution using morphological data sets. The latter three arguments may be considered aspects of the topic of character evolution especially pertinent to including fossil taxa in simultaneous analyses with extant taxa. Below, we address the arguments against including fossil taxa in simultaneous analyses of morphological and molecular sequence data (primarily consisting of arguments against using morphological data in phylogenetic analyses) and go on to discuss some of the specific benefits accrued and obstacles encountered to the study of character evolution when including morphological data from fossil taxa in phylogenetic analyses.

MISSING DATA—A JUSTIFIABLE REASON FOR EXCLUDING FOSSIL TAXA FROM THE PROCESS OF PHYLOGENY RECONSTRUCTION?

One criticism that has been levied against the inclusion of fossil taxa in simultaneous analyses is that fossil taxa may be missing significant amounts of data. For example, Springer et al. (2001: 6242) in part rejected the total evidence approach because "molecular data are usually unattainable for fossils." Perhaps the most serious methodological consequence of including fossil taxa with significant amounts of missing data into an analysis is a weakening of the parsimony criterion, which is strongest when presented with maximum evidence: "the tree that is best corroborated is the tree that best explains (e.g. as homology) all character distributions among all taxa" (Nixon, 1996: 369).

One way in which this weakened test of parsimony may manifest itself is through the wild card taxon phenomenon, in which a taxon (or taxa) with large amounts of missing data may group at numerous different positions on the shortest discovered tree topologies due to its limited distribution of character states (Nixon & Wheeler, 1992). Nixon and Wheeler (1992) noted that the inclusion of wild card taxa in a matrix may result in a significant increase in the number of MPTs and deresolution of the strict consensus of all MPTs. The best solution to such a problem, when encountered following phylogenetic analysis, may be to remove such taxa, provided that the lack of resolution in the position of the problematic taxon can reasonably be attributed to missing data and not at least in part to character conflict. Kearney (2002), in fact, has suggested recognition of three different types of wild card taxa: (1) missing data wild cards, whose instability is entirely caused by missing data; (2) mixed wild cards, whose instability is due both to missing data and character incongruence; and (3) conflict wild cards, taxa whose instability is entirely due to character conflict. Missing data wild cards can be identified by taxonomic equivalent analysis (Wilkinson, 1995), in which fragmentary taxa that are identical with more complete taxa in the characters for which they are coded are removed if an initial analysis shows them to be wild cards, thus eliminating redundancy from the analysis and possibly increasing the resolution of the resultant topologies (see examples in Kearney, 2002).

A more significant barrier to including fossil taxa is the unpredictable effect(s) that missing data can have on a parsimony analysis when character incongruence is encountered, and/or when mixed or conflict wild cards are present. Due to the weakened test of character congruence and the tendency of parsimony to underestimate tree length when large amounts of missing data are present, Nixon (1996: 370) suggested "we should be suspicious when the addition of fossils with large numbers of missing data results in significantly different topologies than when they are excluded." When large amounts of extinction have occurred within a clade, however, significant rearrangements may be expected when fossil taxa are sampled (see examples from Eernisse and Kluge [1993], Wheeler et al. [2004], and Rothwell and Nixon [2006] discussed above), especially when these taxa represent much of a group's diversity. This conundrum may be insoluble, since more complete character data will often be unavailable for fossil taxa. Worse, in combined morphological–molecular sequence data sets, fossil taxa will be coded for a very small proportion of characters, as they will likely be missing the entire molecular sequence partition. In the present study, for example, *Icaronycteris* is coded (with one or more character states) for only about 2% of all

parsimony-informative characters in the combined matrix, and the Green River fossil bat taxon for about 1.3%; *Tanzanycteris*, whose position within or outside of crown group Microchirptera varies, is coded for only about 0.6% of parsimony-informative characters, the smallest proportion among all fossil bat taxa included in the matrix.

Although threshold values for excluding taxa on the basis of the proportion of missing data have been used in some studies (e.g., Rowe, 1988; Benton, 1990; Grande & Bemis, 1998), these have been rejected both logically (e.g., Kearney & Clark, 2003) and on the basis of simulation and empirical studies. Using simulations, Wiens (2003a) showed that it is not the amount of missing data that determines whether the position of a taxon will be unambiguously and correctly resolved but rather whether critical characters are coded for that taxon. This conclusion conforms to a basic principle of phylogenetic systematics: it is only critical character state transformations—synapomorphies—that provide grouping information (Hennig, 1966). Thus, the missing data problem becomes one of too few characters rather than too much missing data, since greater sampling of characters increases the chance that critical characters needed for precise and accurate resolution of a taxon's position will be included in the analysis (Wiens, 2003a). In another simulation study, Wiens (2003b: 309) predicted, "If the fossil taxa can be accurately placed in an analysis of the morphological data alone, they should be accurately placed in the combined analyses as well, regardless of their relative level of incompleteness when the molecular data are added."

Wiens' prediction has been, for the most part, borne out by studies employing pseudofossil analyses. In a pseudofossil analysis, one or more extant taxa are coded for only a subset of characters in the combined data matrix, with all molecular sequence characters and often a portion of the morphological character matrix coded as missing so that the pseudofossil(s) simulate the behavior of a fossil taxon (or taxa) during simultaneous analysis of a real data set (real fossil taxa are excluded). Jordan and Hill (1999), Jordan and Macphail (2003), and Asher and Hofreiter (2006) used an approach in which one taxon at a time was treated as a pseudofossil in combined morphological and molecular sequence data matrices in order to evaluate the behavior of these incomplete terminals in simultaneous analyses of real data sets. In each case, pseudofossils were found to place reliably to the general area of the tree suggested by analyses of the taxa with the full complement of characters, although the results of analyses with pseudofossils were not necessarily identical to analyses in which the full matrix was analyzed. Manos et al. (2007) took a different approach and created pseudofossils by duplicating one extant taxon at a time and randomly eliminating all but 25%, 50%, or 75% of the duplicate's morphological characters or by eliminating all but its organ-specific (i.e., vegetative, floral, or fruit) characters and performing simultaneous analyses. Although the randomly generated pseudofossils were seldom sister to their parent species in the resultant topologies, they did place "in the correct local clade, and neither of the two large clades (engelhardioids or juglandoids) was disrupted" (Manos et al., 2007: 422); "[r]emoval of suites of organ-specific characters did not show appreciably different results" (Manos et al., 2007: 422). In contrast, Springer et al. (2007)—in a pseudofossil study of a combined matrix of placental mammals, in which ordinal or superordinal groups were treated as pseudofossils by eliminating all but the osteological data partition for each taxon in that group—often found profound rearrangements in topology relative to a tree based on molecular sequence data alone. Although Springer et al. (2007) attributed these rearrangements to the inadequacy of the morphological data set, they did not establish whether taxon removal (i.e., taxon sampling) affected the topologies favored by the molecular sequence data partition alone. Thus, the underlying reasons for these rearrangements may have been more complex.

Studies such as those cited above suggest that, as previously noted by Kearney (2002: 370), "[t]he effects of incomplete taxa and concomitant missing character data are not general, but matrix-specific, and depend on the precise distribution of question marks and characters states across taxa"; also see Novacek (1992a). Therefore, taxa with large amounts of missing data should not be considered a priori unsuitable for inclusion in phylogenetic analyses, including simultaneous analyses of morphological and molecular sequence data. The biggest problem that missing data present, then, may be the weakened application of parsimony (however, see Kearney & Clark, 2003). This is especially the case when incongruent data are concentrated in fossil taxa, which may have undesirable effects on the results, such as deresolution of the strict consensus tree or support for misleading topologies. Novacek (1992b: 75) perhaps best summarized the problem: "The kinds of characters preserved, not just the degree of character representation, account for the potential influence of an added taxon. If among the few characters preserved are the combination of primitive and derived states that force relationships in a particular direction, then the included taxa—even when poorly represented—will play a significant role

in the outcome. Of course, the possibility that these incompletely preserved taxa force the wrong outcome, which would be apparent had the taxa been better represented, cannot be eliminated." Unfortunately, at this time, there is no definitive way to differentiate between the correct and the incorrect topology in such a situation.

The molecular scaffold approach may appear to circumvent this problem by building a topology that includes only extant taxa (for which all or most molecular sequence characters can be scored) to serve as a basis for constraints on the analysis of the morphological data, thereby isolating the ambiguity caused by missing data into unconstrained taxa in the morphological partition. Although excluding fossil taxa from the scaffold-building step thus inoculates the molecular partition from the computational and philosophical problems that can be caused by including large numbers of ambiguous cells in a data matrix, the underlying missing data problem is not truly eliminated. This is because excluding fossil taxa from direct phylogenetic analysis also reduces taxon sampling, which in general can negatively impact the accuracy of results (e.g., Zwickl & Hillis, 2002).

CONVERGENCE

According to Givnish and Sytsma (1997: 56), there are four mechanisms that can result in homoplasy: "evolutionary convergence, recurrence, transference, and character misclassification [bold emphasis removed]." Evolutionary convergence or parallelism (cf. Wiens et al., 2003; Desutter-Grandcolas et al., 2005) of form may result when similar morphological solutions to similar selective pressures are discovered. There appears to be a perceived notion among some workers that convergence of morphological form is so pervasive and misleading that it is justifiable to either (1) simply exclude morphological data from the construction of phylogenetic hypotheses or (2) regard morphological data as suspect when topological conflict with molecular sequence data occurs (Hedges & Sibley, 1994; Hedges & Maxson, 1996; Givnish & Sytsma, 1997; Eick et al., 2005). There is no denying that convergence of form is a widespread feature of evolution and that it has occurred at many scales and in many taxa through geologic time (some examples include ichthyosaurs and dolphins, rudist bivalves and reef-building corals, some placental and marsupial mammals, the mangrove habit in plants, etc.). As noted by Wiens et al. (2003: 501), "Convergence is a critical issue in systematics because it can potentially mislead phylogeny reconstruction methods, for example, causing analyses to group distantly related organisms that share similar habitats."

Of particular concern are cases in which convergence has potentially led to correlated patterns of evolution in suites of functionally related morphological characters included in an analysis (see, for instance, Wiens et al., 2003). In such cases, however, precise character definition (i.e., hypotheses of homology) and careful morphological study have reduced and will continue to reduce the occurrence of this problem (that is, the fourth class of homoplasy given by Givnish & Sytsma [1997]: character misclassification). One approach is atomization and coding of such morphological complexes, which can highlight differences in apparently convergent morphologies. Bruneau (1997), for example, contrasted the character complex of pseudo-tubular corollas, convergent among hummingbird-pollinated species of the legume *Erythrina* L. (a legume), with the atomized corolla characters detailing specific aspects of size and morphology. Consideration of pseudo-tubular corollas as a single presence-absence character would have led to a relatively uninformative hypothesis of homoplasy due to multiple origins, while breaking the complex pseudo-tubular corolla character into several atomized petal characters allowed for the detection of convergence in a single aspect of the corolla morphology (see also Luckow & Bruneau, 1997). Nixon (1996: 368) pointed out that it is especially important that homology assessments be approached carefully with fossil taxa because "poorly preserved fossils may have a higher likelihood of being misunderstood and therefore incorrectly scored for those characters that are **not** missing [boldface in original]." Thus, in some situations in which structures are poorly understood, perhaps fossil taxa should be coded using different homology assessments and the results of analyses compared or the fossil taxa should be excluded from analysis altogether. Despite this last caveat, the problem of morphological data being seriously compromised by evolutionary convergence may be overstated.

There have been demonstrated cases in which convergence of morphological characters may have produced misleading results. An interesting example was provided by a study from Gatesy et al. (2003) on the relationship of crocodylians to the extant gavial (*Gavialis gangeticus* (Gmelin)), in which results of analyses from a morphological data set (modified from Brochu, 1997), molecular sequence data sets, and a combined data set for fossil and extant crocodilians were compared. Analysis of the morphological data alone with and without fossil taxa suggested that the extant gavial was the most basally diverging lineage of the extant crocodylian groups; in contrast, molecular sequence data suggested that the gavial was in a derived position in the tree, sister to the false gavial

Tomistoma schlegelii Müller. When the data sets were combined, the strict consensus of the resultant MPTs was congruent with the molecular results for extant taxa, suggesting that the extant gavial is an atavistic taxon.

Although Gatesy et al. (2003) could have chalked up these results to the unsuitability of the morphological data, beset by convergence, to discover the proper position of the extant gavial among crocodylians, they instead tested the morphological data for secondary signals.

Secondary signals (Nixon & Carpenter, 1996) or hidden support (Gatesy et al., 1999) are patterns present in a data partition that are masked when that partition is analyzed alone—reflecting a primary signal—but which may manifest themselves as character support for clades conflicting with the primary signal for the partition when analyzed in combination with other data partitions. When data sets that independently produce competing results (i.e., have different primary signals) are combined and analyzed together, secondary phylogenetic signals common to two or more data sets may even lead to novel hypotheses of phylogeny not expressed in any individual partition (see Barrett et al., 1991; Nixon & Carpenter, 1996; Gatesy et al., 1999). In other words, during simultaneous analysis, "[t]he peculiarities of each data set are cancelled out by the unique peculiarities of the others, and the remaining common signal emerges" (Gatesy et al., 1999: 301). Gatesy et al. (2003) tested for hidden support for the derived extant gavial hypothesis in their morphological character data set using partitioned hidden branch support (PHBS; Gatesy et al., 1999). PHBS is the difference between the branch support values for a given data partition and a given node in combined and partitioned analyses (Gatesy et al., 1999); a "positive PHBS score for a particular data set indicates a secondary phylogenetic signal for the relationship of interest that emerges in simultaneous analysis" (Gatesy et al., 2003: 407). Using PHBS, Gatesy et al. (2003) found hidden morphological support for nodes linking the extant gavial to the extant false gavial and the Crocodilynae (crocodiles) to the gavial and false gavial in the combined morphological and molecular sequence topology for extant taxa. This support was increased by the addition of fossil taxa. In a more recent expanded analysis of crocodylians that included increased taxon and character sampling, Gatesy et al. (2004: 347) found "11 groups that emerged in the supermatrix analysis" that "were not implied by any combination of trees supported by separate analyses of the 17 data sets in the supermatrix," thereby revealing the secondary signals that manifested themselves during simultaneous analysis. Similarly, secondary signals must be at work in the simultaneous analysis of Chiroptera presented here, because the two major clades of bats resolved during simultaneous analysis are concordant with the primary morphological but not the primary molecular sequence signal (Fig. 1), suggesting that secondary signals in the molecular sequence partition may support the traditional Megachiropteran and Microchiropteran groupings.

The discovery of these secondary signals is only possible through simultaneous analysis and is an important advantage of this approach over the molecular scaffold approach. For instance, in the study from Asher et al. (2005a) on the extinct insectivorous mammal *Centetodon*, simultaneous analyses of molecular sequence and morphological data indicated that *Centetodon* was more derived than the Caribbean endemic insectivore *Solenodon*, whereas a molecular scaffold analysis failed to decisively resolve the position of *Centetodon* relative to *Solenodon*. Asher et al. (2005a: 919) considered this ironic since "a basal position for *Solenodon* within Holarctic lipotyphlans [mammalian insectivores] results from the molecular signal responsible for the molecular scaffold, and not from the influence of the morphological data. Analyzed alone, morphological data weakly support *Erinaceus* as basal" within the clade. Thus, the molecular scaffold actually obscured both the molecular sequence signal as well as the signal common to both data sets when analyzed together.

Furthermore, the molecular scaffold approach may tend to force more conflict into the data set that is analyzed on the scaffold, depending on the constraints used. This creates a self-fulfilling prophecy whereby the morphological data contain larger amounts of homoplasy—sometimes explained ad hoc as evidence of convergence (see also Luckow & Bruneau, 1997)—relative to the results of a morphological analysis alone, and sometimes relative to the results of a simultaneous analysis. The bat data set presented here is an interesting example of this phenomenon. The morphological data gained 132 steps relative to the MPTs for the morphological partition alone, and 35 or 48 steps relative to the MPTs for the simultaneous analysis when analyzed under a strict molecular scaffold (compare analyses 1a and 3b to analysis 4, Table 2). The overall CI and RI for the combined analysis (3b), the combined analysis with Yinpterochiroptera–Yangochiroptera constraints (3c), and the strict molecular scaffold (4) are nearly identical for the combined data set (Table 2). However, the distribution of homoplasy between partitions is shifted from the molecular sequence to the morphological characters as the data are put under constraints that are progressively more favorable to the primary

molecular sequence signal. The CI and RI become progressively lower (e.g., more homoplasious) for the morphological data and higher for the molecular sequence data (Table 2). The change in the CI and RI values for the morphological partition is also greater, reflecting the fact that, although the absolute difference in the number of steps for each partition is similar between the most and least parsimonious analyses for that partition (35 or 48 steps for morphology, 20 or 33 steps for molecular sequence), proportionally more steps are forced into the morphological partition in less parsimonious topologies for that partition than for the molecular sequence data set, because the molecular sequence data set is much larger (Table 2).

Eick et al. (2005) recently took assumptions about convergence in morphological characters to an extreme and analyzed fossil-extant bat relationships on a molecular scaffold using only morphological characters that were nonhomoplasious when mapped on a topology suggested by molecular sequence data because "the exclusion of a significant amount of homoplasious characters can potentially alter the conclusions reached" (Eick et al., 2005: 1872) and "the high level of parallel evolution when using morphological characters is problematic" (Eick et al., 2005: 1879). Interestingly, they did not remove homoplasious molecular sequence characters from their analyses, although homoplasy was present in the molecular sequence data (the ensemble RI was less than one for each data set when analyzed using parsimony). Molecular sequence data are not necessarily exempt from evolutionary convergence. Lee (1997) noted that biologists understand functional morphology well and are adept at recognizing and articulating the adaptive significance of some aspects of the morphologies of organisms. Conversely, Lee (1997) questioned whether the young discipline of functional molecular biology is yet as capable of recognizing adaptive convergence in sequence data. In fact, recent work has demonstrated the occurrence of evolutionary convergence at the molecular level (e.g., Bull et al., 1997; Zhang & Kumar, 1997; Cuevas et al., 2002; Zakon, 2002; Protas et al., 2006). While we agree with some authors (e.g., Hedges & Maxson, 1996) that molecular sequence data may be less prone to similarity caused by evolutionary convergence or parallelism, molecular sequence data are also subject to homoplasy from recurrence (random mutation leading to noisy homoplasy; see Wenzel & Siddall, 1999) and transference (horizontal gene transfer), as well as character misclassification (e.g., due to misalignment). Because of these four factors, the amount of homoplasy in sequence data may be as great as or greater than that in morphological data (e.g., Sanderson & Donoghue, 1989; Baker et al., 1998). Thus, it should be borne in mind that sequence data do not have special immunity from homoplasy—as powerfully evidenced by "the fact that different genes yield different phylogenies" (Lee, 1997: 394)—and, as in morphological data, this homoplasy is potentially misleading.

Only additional tests of a phylogenetic hypothesis through the addition of more taxa and more characters can be the ultimate arbiter of the robustness of a suggested set of relationships. As perhaps best summarized by O'Leary et al. (2003: 861), "Without insights into some yet undiscovered law of nature, there is no particular reason to think that a functional, developmental, or ecological explanation for homoplasy is a better explanation of covariation than is synapomorphy. Simply proposing such generalities does not condemn characters to being phylogenetically uninformative."

THE WEIGHTING PROBLEM

It has been argued that in a simultaneous analysis of morphological and molecular sequence data, the large amounts of molecular sequence data will simply overwhelm (or swamp) the signal of the morphological data set. For example, Alvarez et al. (2000: 184) argued that the morphological and molecular sequence data sets they used to reconstruct the phylogeny of a family of sponges "are unequally sized (95 parsimony-informative molecular characters vs. 16 parsimony-informative morphological characters) so that the molecular signal, which is different, will swamp the morphological signal. Therefore, a simultaneous analysis (e.g. one including both types of data) was considered inappropriate." Some might argue that because morphological transformations might be presupposed to involve more complex underlying mechanisms than transformations among nucleic acid bases, perhaps morphological data partitions should be upweighted with respect to molecular sequence data partitions. This assumption may be analogous to that employed in some analyses of molecular sequence data sets: "Within that paradigm of probabilistic assumptions, it is often argued that transitions are more probable (i.e., observed more frequently) and should therefore deserve a lower cost...than transversions. However, no consensus has been reached for the appropriate cost ratio, and an arbitrary choice is required" (Frost et al., 2001: 354). The arbitrariness in weighting schemes may be the problem alluded to by Springer et al. (2004: 436) when they wrote, "One potential difficulty with combined analyses is that they fail to address the weighting problem posed by including

molecular and morphological data in the same data matrix."

Empirical studies of combined morphological and molecular sequence data sets do not bear out the assumption that large numbers of molecular sequence data will necessarily overwhelm the signal present in less numerous nonmolecular sequence data when all characters in a combined matrix are weighted equally (e.g., Omland, 1994; Mattern & McLennan, 2004; Wahlberg et al., 2005). This observation ties into the idea of secondary signals discussed above: when the data are combined, a common signal present in both data sets may be expressed that is not evident in either data set (or one of the data partitions) when analyzed alone (see similar argument made by Barrett et al., 1991). Obviously, the bat data set analyzed here exemplifies this: although the molecular sequence data set is much larger than the morphological data set, even when only parsimony-informative characters are considered (202 morphological, 3533 molecular sequence, or a ratio of parsimony-informative molecular sequence to morphological characters of over 17:1), the combined data set produces major groupings that are congruent with the morphological but not the molecular result, clearly showing that the morphological signal is not completely swamped by the signal from the molecular sequence data set but rather contributes to the structures of the most parsimonious topologies.

A study done by Baker et al. (1998) specifically examined the distribution of morphological and molecular sequence character support for nodes in trees generated by equal-weights parsimony applied to combined data sets. These authors compared the partitioned Bremer support (PBS; Baker & DeSalle, 1997; Lambkin et al., 2002) values from 10 previously published combined morphological–molecular sequence data sets (five additional combined data sets in their study featured non-DNA sequence molecular data) with ratios of parsimony-informative morphological to molecular sequence characters ranging from 0.08–0.33; results of the incongruence length difference (ILD) test (Farris et al., 1995a, b) for these data sets indicated that in five of 10 cases, there was significant incongruence between morphological and molecular sequence data partitions. When PBS values were summed across nodes for each of the studies, the morphological data provided proportionally slightly more support (relative to the number of parsimony-informative characters in each partition) to the topology resulting from simultaneous analysis than molecular sequence data for nine of the 10 data sets. Notably, the morphological data were also, in nine of 10 instances, less homoplasious than the molecular sequence data sets on the topology(ies) resulting from simultaneous analyses as measured using the CI of parsimony-informative characters for each partition; Baker et al. (1998) suggested that this may account for the greater impact of the morphological partitions relative to their sizes. Similarly, Lee (2005) combined morphological and molecular sequence data in analyses including extant and fossil squamates (e.g., lizards, snakes, amphisbaenians, and extinct relatives) and examined PBS values. The results of the combined analyses (under parsimony and Bayesian criteria) were mostly congruent with the result of the morphological analysis alone (under the parsimony criterion). Furthermore, "PBS values [from the parsimony analysis] revealed that the morphological signal (although weak) is still stronger than the molecular signal" (Lee, 2005: 229), even though there were more parsimony-informative molecular sequence characters than morphological characters. (See also Wortley & Scotland, 2006, for a more recent study using different methods.) Baker et al. (1998) have suggested, in fact, that the larger data partition in a combined matrix should have more influence on the results of a phylogenetic analysis—for the result to be otherwise would cast doubt on the quality of the larger data set.

Another argument against the necessity of weighting—character independence—has been effectively addressed by a number of authors (e.g., Kluge & Farris, 1969; Kluge, 1989; Nixon & Carpenter, 1996). Nixon and Carpenter (1996), who made a thorough argument for employing equal weights in a parsimony analysis a priori, addressed the issue of weighting data partitions using an appeal to character independence. Characters chosen for inclusion in a cladistic analysis are hypothesized to be independent of one another—they are logically independent, or assumed to evolve independently (Farris, 1983; Kluge & Wolf, 1993; Nixon & Carpenter, 1996). If characters are assumed to be independent from the outset, as they are in a parsimony analysis, then there is no more justification to apply weights among different data partitions than to apply differential weights to characters within a data partition. Thus, the perceived greater complexity of morphological characters need not be used as justification for upweighting them relative to molecular sequence characters.

Some have employed differential costs for morphological and molecular transformations, however, without resorting to completely arbitrary weighting schemes. In the context of simultaneous parsimony analyses including both morphological and molecular sequence data, this has been done using POY (Wheeler et al., 1996–2003), in which differential costs can be assigned to transitions, transversions, insertion and deletion events, and morphological

transformations, and direct optimization is employed during analysis (Wheeler, 1996, 2003). In empirical studies in which POY is applied to simultaneous analyses of morphological and molecular sequence data with fossil and extant taxa, measures such as ILD metrics and a topological index have been used to choose the optimal weighting scheme(s) from those applied to the data (Giribet et al., 2002; Asher et al., 2003, 2004).

Finally, the molecular scaffold approach does not overcome the weighting problem and, in fact, appears to resurrect a widely recognized weighting problem that arises when topology is used as a proxy for character matrices: although an explicit weighting scheme is not imposed, an implicit weighting scheme is employed (e.g., Barrett et al., 1991). The molecular scaffold implies differential weights for the characters used to construct the molecular scaffold topology relative to the characters in the matrix analyzed under the scaffold constraints, and these weights are node specific. The morphological characters are effectively weighted to zero for extant taxa where nodes are constrained (e.g., the nodes cannot be overturned by morphological characters), whereas the molecular sequence characters, where applicable, are effectively weighted to zero at unconstrained nodes. Thus, far from circumventing the weighting problem, the molecular scaffold generates a unique weighting problem of its own.

Whether or not one wishes to view the molecular scaffold approach under the rubric of a weighting scheme, the implicit assumptions built into the approach are contradictory. At nodes that are constrained, the molecular sequence data are considered reliable, whereas at unconstrained nodes they are not. Thus, in a strict molecular scaffold—in which all nodes suggested by the molecular sequence data are constrained (e.g., O'Leary & Geisler, 1999; Sánchez-Villagra et al., 2003; Roca et al., 2004; Asher et al., 2005a; Lee, 2005; Teeling et al., 2005; Xiang et al., 2005)—the sequence data are assumed to provide a reliable estimate of phylogeny at all nodes where they are informative, whereas the morphological data only contribute to the structure of the tree where molecular sequence data cannot be applied. In a semi-strict molecular scaffold—in which only some nodes recovered by the molecular sequence data are constrained (e.g., Springer et al., 2001; Lee, 2005; Kay & Cozzuol, 2006; Magallón, 2007; Manos et al., 2007)—the sequence data are only allowed to contribute to the tree topology at nodes that meet a given criterion (for instance, have certain support values: cut-off values for nodes in semi-strict molecular scaffolds have ranged from 70% [Lee, 2005] to 90% [Springer et al., 2001] in previous studies), whereas the morphological data contribute structure only at nodes that do not meet the threshold criterion or where sequence data do not apply. Thus, an implicit and contradictory judgment about the value and reliability of each data partition for phylogeny reconstruction is built into the molecular scaffold approach—molecular sequence data are generally favored, whereas morphological data have value only when evidence from sequence data is poor or nonexistent.

CHARACTER MAPPING AND THE STUDY OF MORPHOLOGICAL CHARACTER EVOLUTION

Regardless of their effect on tree topology or the method used to include them in an analysis, fossil taxa are undoubtedly very significant in the application of phylogenetic hypotheses, specifically interpreting morphological evolution within and across monophyletic groups. In fact, it seems safe to suggest that the study of character evolution across major organismal groups such as the seed plants and amniotes must incorporate fossil taxa to be relevant. This is because so much of the diversity that links extant end points—or even stands on its own as a major component of the evolutionary history of these groups—is missing in analyses that incorporate only extant taxa. In such cases, the hierarchical nature of evolution coupled with the removal of diversity through extinction may have the power to obscure relationships and morphological character evolution in a way that can only be addressed through reference to the fossil record (e.g., Wheeler, 1992; Cobbett et al., 2007).

Before discussing the potential benefits and difficulties of studying character evolution using a phylogenetic hypothesis that includes fossil taxa, it is necessary to address an old argument that continues to persist in the literature: that it is circular to map a character on a phylogeny when that same character is incorporated into the matrix on which the phylogeny is based. Thus, the argument goes, if one wishes to explore morphological character evolution, molecular sequence data should be used to construct a phylogeny, and morphological data should be mapped on the phylogeny after the fact, wherein they can be used to trace the evolution of morphological traits (Armbruster, 1992; Hedges & Maxson, 1996, 1997); or, more generally, characters to be mapped should be removed from the matrix when building a phylogeny so that that phylogeny is independent of the mapped characters (e.g., Springer et al., 2001). If inclusion of morphological characters in matrices and subsequent mapping of those same traits on the resultant trees were circular, this would pose a grave problem for paleontologists. How best to study morphological

character evolution in a phylogenetic context when morphological characters are usually the only source of information about fossil taxa that can be harnessed to build phylogenies including those taxa?

The most fundamental assumption of the independence or circularity argument appears to be that the study of character evolution should be independent of tree building, or that the character(s) under consideration in proposing a specific evolutionary hypothesis must be independent of the construction of the tree used to test that evolutionary hypothesis (Coddington, 1988; Brooks & McLennan, 1991). However, there is no logical reason that a phylogeny should be independent of the characters to be mapped on it. If the characters to be mapped are assumed to be among the class of characters that are suitable for phylogeny reconstruction, they should not be independent of phylogeny, and there is no circularity in mapping those same characters onto the phylogenetic topology or topologies on which they have been optimized during the tree search (e.g., Deleporte, 1993; Kluge & Wolf, 1993). This is because all characters coded for a terminal coexist within the same taxon and are hypothesized to be the result of the evolutionary history of that taxon and informative with respect to phylogeny. Thus, independence of a phylogeny from a character to be mapped on that phylogeny is not really achieved by removing a phylogenetically informative character from the analysis: although the removed character is independent of the mechanics of building the phylogeny, it is not independent of the true phylogeny or evolutionary history of the taxa under consideration in the analysis, which we presume to exist independently of our ability to correctly recover it. In fact, the unintended consequence of character removal in order to avoid circularity may be to make phylogenetic hypotheses themselves less robust, because evidence pertinent to evolutionary history is being ignored (see Kluge, 1989; Nixon & Carpenter, 1996), which can impact the tree topologies recovered.

That argument addressed, inclusion of fossil taxa among the terminals in a phylogenetic analysis has great potential for elucidating the evolutionary histories of individual aspects of whole organisms. Applications include: (1) determination of the sequence of character transformation; (2) insight into the timing of evolutionary events; and (3) inference of unknown aspects of the morphology (e.g., due to incomplete preservation) or behavior of extinct organisms and especially testing of evolutionary hypotheses.

SEQUENCE OF CHARACTER EVOLUTION

Inclusion of fossil taxa as terminals on trees can help to bridge gaps in our understanding of morphological character evolution as viewed solely from the present. Fossil taxa provide a broader sampling not only of morphologically but also of temporally diverse taxa, and thus are actual data points representing samples taken from across time at different stages in the evolutionary history of a group (Cobbett et al., 2007). As such, their inclusion as terminals in phylogenetic analyses may alter the perceived sequence of character state transformations in a group regardless of whether their inclusion significantly alters tree topology.

A simple situation in which a fossil taxon or fossil taxa can impact understanding of character evolution is when a fossil taxon or fossil taxa are intercalated among the synapomorphies along an internode that would be unbroken if only extant taxa were considered. Intercalation of such taxa may indicate the sequential evolutionary order of synapomorphies that otherwise cluster at the same node when only extant taxa are considered. For instance, in a simultaneous analysis of morphological and molecular sequence data for extant and fossil taxa within the angiosperm order Saxifragales by Hermsen et al. (2006), inclusion of the Cretaceous flower and fruit taxon *Microaltingia* Zhou, Crepet & Nixon indicated that the evolution of unisexuality and loss of the corolla preceded the evolution of winged seeds and decurrent stigmas in the stem group leading to the clade including extant *Cercidiphyllum* Siebold & Zucc. (katsura) and Altingiaceae (sweet gum family). Thus, inclusion of *Microaltingia* broke up a series of synapomorphies that would otherwise have occurred together at the node subtending the extant clade, assuming the same topology and character optimizations if *Microaltingia* were removed (Hermsen et al., 2006). Horovitz (1999a) compared the results of simultaneous analyses of morphological (primarily skeletal) data and molecular sequence data from platyrrhines (New World monkeys) in which the relative relationships among the extant taxa were the same with and without inclusion of the fossil taxa. In at least three specific instances, inclusion of the fossil taxa broke up clusters of synapomorphies, introducing "a stepwise appearance of different characters along the phylogeny, that would seem to appear in larger clusters of synapomorphies in the phylogeny composed of Recent taxa only" (Horovitz, 1999a: 24). One instance involved the subfamily Callitrichinae (marmosets and tamarins), which was supported by eight synapomorphies in the extant-only analysis; these character transformations were spread among four separate nodes when fossil taxa were included. Horovitz (1999a: 26) suggested that the breakup of the synapomorphy cluster by the fossil taxa "shed some light on the process that was presumably a consequence of reduction in body size."

Inclusion of fossil taxa may also change the perceived utility of characters to provide grouping information. Character states that are autapomorphic (parsimony-uninformative) when only extant taxa are considered may turn out to be synapomorphies when fossil taxa are included (Donoghue et al., 1989). Horovitz (1999a) provided a good example of this in her comparison of topologies recovered from simultaneous analyses for extant platyrrhines with and without fossil taxa. In that example, *Callimico goeldii* Thomas (Goeldi's marmoset) is offset by four autapomorphies in the tree including only extant taxa; two of these autapomorphies became synapomorphies for *Callimico* and two fossil taxa (*Patasola magdalenae* Kay & Meldrum and *Carlocebus carmenensis* Fleagle) when the fossil taxa were included in the analysis (Horovitz, 1999a). Alternatively, character transformations that appear to be unambiguous synapomorphies when only extant taxa are considered may become homoplasious when more diversity is sampled (Donoghue et al., 1989). The Gatesy et al. (2003) simultaneous analyses of crocodylians with and without fossil taxa provide examples of this. As they noted, "A more complete sampling of taxa [inclusion of fossil taxa] uncovered additional homoplasy, revealed uncertainties in character optimizations, and ultimately overturned hypotheses of homology that were based solely on the extant biota" (Gatesy et al., 2003: 412). As a specific example, among extant taxa alone, presence of "rectangular dorsal midline osteoderms" was an unequivocal synapomorphy for *Tomistoma schlegelii* (false gavial) and *Gavialis gangeticus* (gavial), whereas "[t]he combined evidence topology [including fossil taxa] implied that square/equant osteoderms instead were independently derived from rectangular osteoderms within Alligatoroidea and Crocodylinae" (Gatesy et al., 2003: 412).

Fossil taxa may also help to establish the polarity within characters present in a clade but inapplicable in the most closely related extant clades; this may be especially true for groups offset by many characters not represented in their nearest living relatives (Donoghue et al., 1989). Illustrative of this phenomenon in the context of simultaneous analysis of morphological with molecular sequence data is that of the relationship of the gavial to the remainder of extant crocodilians (Gatesy et al., 2003, introduced above). Simultaneous analysis of morphological and molecular sequence data including both extant and fossil crocodilians resolved some fossil taxa along the stem lineage leading to extant crocodilians, their presence clarifying and, in some cases, overturning the polarity of character states within characters for the ingroup when compared to the analysis of data from extant taxa alone. For instance, when only extant crocodilians were included in the simultaneous analysis of morphological with molecular sequence data, character state polarity could not be established in the occlusal pattern of dentary teeth in crocodilians because the character was inapplicable in the outgroup (Aves, birds); however, when fossil taxa representing more closely related outgroups were included, a clear polarity was established, suggesting Alligatoroidea (alligators) have the plesiomorphic condition. This occurred because the nearest outgroup (Aves, birds) for the extant crocodilians alone was coded as inapplicable for 42% of the morphological characters present within the extant crocodilians, and thus the fossil taxa proved more relevant in analyzing character evolution within the least inclusive clade including all extant crocodilians (Gatesy et al., 2003).

Fossil taxa may also help to establish a single preferred optimization sequence of character states that have multiple equally parsimonious optimizations when only extant taxa are considered, reducing ambiguity byincreasing taxon sampling density. For an example from crocodylians of how a suite of characters was differentially optimized (in terms of gains and losses) when fossil taxa were included or excluded from phylogenetic analysis, see Gatesy et al. (2003).

Finally, fossil taxa may add entirely novel characters or variations within characters to an analysis, providing information that we would have been completely ignorant of were they not included. For instance, inclusion of fossil penguins in an analysis of morphological and molecular sequence data for all extant penguins by Clarke et al. (2007) demonstrated that the ancestral beak morphology for penguins is "[a]n elongate, powerfully constructed beak unknown in extant penguins" (Clarke et al., 2007: 11550).

TIMING OF CHARACTER EVOLUTION

Another, perhaps presently underexploited, aspect of character mapping in the context of a phylogeny is to bracket the timing of character transformations. While determining a maximum limit on the time of appearance of a given synapomorphy by reference to the fossil record is controversial—as it is wrapped up with determining ancestry (Hermsen & Hendricks, 2007) and is impeded by the fact that the true time of the first appearance of a fossil taxon (and thus the characters it bears) cannot be confirmed simply through a literal reading of the fossil record—determining the minimum age of a synapomorphy is straightforward. It has long been recognized that the minimum age of a clade can be estimated by reference to the oldest of the descendants of the most recent common ancestor of that clade (e.g., Hennig, 1966);

this technique for estimating the minimum age of a clade has been exploited using various permutations of the same basic idea, such as comparison of the age of a clade to that of its sister taxon or ghost lineage analysis (Norell, 1992, 1993), or minimum age node mapping (Crepet et al., 2004: also see Hermsen & Hendricks, 2006). The minimum age of the most recent common ancestor of a clade is also the minimum age of the synapomorphies that define that clade, unless one or more of the synapomorphies is demonstrably older. Thus, mapping of characters onto a phylogeny and consideration of the minimum ages of the nodes on a cladogram can allow one to place minimum ages on synapomorphies as well. The bat phylogeny does not provide a particularly compelling example of this, since some of the characters of most interest that are not preserved in the fossil taxa (e.g., laryngeal echolocation) cannot be mapped unambiguously on the resultant trees, or too many topologies exist to make accurate age interpretations; however, in some of the trees from the unconstrained simultaneous analysis (analysis 3b, Fig. 1) and from the molecular scaffold analysis (analysis 4, Fig. 2), at least one Eocene fossil bat taxon is nested within a clade of bats characterized in part by laryngeal echolocation. Therefore, minimum age node mapping performed concurrently with character mapping on these trees suggests that laryngeal echolocation had evolved by the Eocene, an inference that has also been made through structural analysis of the fossils (e.g., Simmons & Geisler, 1998).

Hermsen et al. (2006) provided another example of minimum age node mapping overlain on character mapping in their simultaneous analysis of the angiosperm clade Saxifragales (introduced above in the section "Sequence of Character Evolution"). In that example, which occurs only in two of eight MPTs, minimum and maximum ages for the timing of evolution of dorsifixed anthers in the clade including the extant families Pterostemonaceae and Iteaceae (Virginia willow family) were inferred on the basis of traits present in the fossil flower and fruit taxon *Divisestylus* Hermsen, Gandolfo, Nixon & Crepet (two species of which were included as terminals in the analysis) as well as fossil pollen that was not directly included as a terminal in the analysis. The synapomorphy of dorsifixed anthers was mapped on the branch immediately descendant to the node where the species of *Divisestylus* attached. Because one species, *D. brevistamineus* Hermsen, Gandolfo, Nixon & Crepet, had been documented to have had basifixed anthers (Hermsen et al., 2003)—suggesting that the dorsifixed condition had not yet evolved—it was used to supply a maximum age of about 90 million years ago (Ma) on the appearance of dorsifixed anthers. Dispersed diporate pollen, a synapomorphy for Iteaceae (*Choristylis* Harv. and *Itea* L.), mapped at the node descendant to the node supported by dorsifixed anthers, provided a minimum age of about 50 Ma, the approximate time of first appearance of this pollen in the fossil record (Moss et al., 2005). Thus, it was suggested that a transition from basifixed to dorsifixed anthers occurred somewhere between about 90 and 50 Ma.

Hermsen and Hendricks (2007) formalized a methodology for applying minimum and maximum ages (relative or numerical) on the appearance of synapomorphies that are mapped on a cladogram that includes fossil taxa as terminals. While methods for inferring the timing of first appearance of synapomorphies with reference to fossil taxa included directly in cladistic analyses have not often been explicitly employed by overlaying minimum age node mapping and character mapping on a cladogram, the timing of first appearance of a trait has been inferred using similar logic. For instance, inference of the existence of a given trait at a given time might be made when optimization suggests that trait was present in a fossil taxon in which it cannot be directly observed (see discussion below on inference, ambiguity, and hypothesis testing), thus providing a minimum age for the appearance of that synapomorphy.

INFERENCE, AMBIGUITY, AND HYPOTHESIS TESTING

One great promise of phylogenetics as applied to the fossil record is the ability to test structural, functional, or behavioral hypotheses by inferring the presence or absence of particular structures, behaviors, or functional features in fossil taxa through optimization of these attributes on phylogenetic trees; this is especially important when these attributes are unlikely to be preserved or are unpreservable in the fossil record. As pointed out by Bryant and Russell (1992) and Witmer (1995), a phylogenetic bracket (to use Witmer's term) is necessary in order to unambiguously infer the presence or absence of traits in a fossil taxon in which these traits cannot be observed; in other words, taxa that are unambiguously known to lack or possess a feature of interest must flank the taxon in which the trait is unknown in order to unambiguously infer that trait's absence or presence in that taxon (e.g., to unambiguously optimize the character state transformation at the node where the fossil taxon attaches to the tree: see also discussion in O'Leary, 2001). O'Leary (2001), for instance, was able to infer whether basal Cetacea (whales) had hair (extant cetaceans do not have hair; O'Leary, 2001) using a simultaneous analysis of morphological and molecular sequence data for cetaceans and artiodac-

tyls (even-toed hoofed mammals). Mapping of the character for hair (with presence/absence states) onto two of 33 MPTs unambiguously indicated that the fossil taxa *Ambulocetus* Thewissen, Hussain & Arif and *Pakicetus* Gingrich & Russell (extinct basal whales) were hairless, since they were bracketed by extant taxa, the hippopotamids and extant cetaceans, which lack hair (O'Leary, 2001: figs. 6b, 6c). The extinct mammal group Mesonychia was reconstructed as either hairless or hairy depending on where the group was resolved in the MPTs.

Phylogenetics may be limited as a tool for inferring behavioral or functional (or even unpreserved structural) features of extinct taxa, however. O'Leary (2001) provides several examples of this in her total evidence analysis of cetaceans and artiodactyls. Structural evidence has been used to hypothesize that, for instance, basal whales were capable of terrestrial quadrupedal locomotion and of processing sound under water (see citations in O'Leary, 2001). However, O'Leary (2001) was unable to unambiguously infer whether basal whales were capable of these behaviors through optimization of behavioral characters on phylogenetic trees. She suggested that direct fossil evidence—when available—can provide the ultimate corroboration (or refutation) of a functional or behavioral hypothesis, and, where direct evidence is unavailable, indirect evidence (for instance, interpretation of an organism's habitat) could be utilized to address some questions that cannot be addressed successfully through character optimization. For instance, the hypothesis that early whales were quadrupedal was suggested by observation of the breadth of the sacro-iliac joint in the extinct whale *Ambulocetus* and also inference about the breadth of the sacro-iliac joint in *Pakicetus* through character optimization on phylogenetic trees (O'Leary, 2001: fig. 6). The behavior of quadrupedal locomotion was, however, ambiguously optimized for these taxa; thus, the hypothesis of terrestrial quadrapedal locomotion in early whales cannot be corroborated through character optimization. In this case, direct fossil evidence could theoretically provide a solution: "if footprints of a pakicetid or ambulocetid were found" (O'Leary, 2001: 501), the behavioral hypothesis of quadrupedal locomotion in these early whales could be corroborated. In the case of adaptation to hearing under water—where presence of a pachyostotic bulla in the ears of extinct basal whales suggests they were capable of hearing underwater—optimization is ambiguous and direct fossil evidence about hearing capabilities does not (and likely cannot) exist. Thus, "we are left with character correlation argument only or an inference from design or environment based on the characteristics of organisms that happen to be alive" (O'Leary, 2001: 501).

For bats, extrapolation from structural features has been used to suggest that the extinct taxa *Archaeonycteris*, *Hassianycteris*, *Palaeochiropteryx*, and *Tanzanycteris* were capable of laryngeal echolocation (e.g., Simmons & Geisler, 1998; Gunnell et al., 2003). While mapping of the echolocation character on the topologies based on unconstrained combined data is equivocal for presence or absence of this behavior in these taxa in most instances (except *Tanzanycteris*, which can be inferred as capable of the behavior in eight of 16 trees; Fig. 3), hypothesizing that they were capable of laryngeal echolocation on the basis of structural features does not add extra steps to the MPTs. Such an interpretation instead imposes a preferred approach to mapping the echolocation character such that its presence would support the node immediately descendant to the node at which Megachiroptera attaches to the tree, although presence of this behavior is not directly testable using the fossil record. Certainly, the examples from bats and whales expose one of the great weaknesses of phylogenetics for addressing aspects of evolution that go unrecorded in the fossil record: "While questions about stem taxa to major clades are often some of the most interesting, they can expose areas where it is very difficult to test functional and behavioral inferences in fossils with cladistic character data" (O'Leary, 2001: 501) due to the lack of extant taxa that can serve as phylogenetic brackets that allow for unambiguous optimization of intangible features.

When structural (or other) evidence contravenes the optimization of a behavioral character on a phylogeny, the problem of functional and behavioral inference becomes even more complex. Simmons and Geisler (1998), for instance, have argued that structural evidence suggests that *Icaronycteris* was capable of laryngeal echolocation (see similar discussion of echolocation in *Icaronycteris* by Novacek [1987], Gunnell & Simmons [2005], and Simmons [2005a]), although mapping of the laryngeal echolocation character on the more recent most parsimonious topologies from morphological data (Gunnell & Simmons, 2005) and the tree resulting from the combined analysis here (Fig. 3) suggest that it was not. When structural features and character mapping disagree, one must choose which is preferable, or whether the evidence is equivocal. In the bat example, therefore, one must decide whether *Icaronycteris* should be coded as missing (?) for the presence of laryngeal echolocation (as it is in the matrix of Gunnell and Simmons, 2005), or whether it should be assumed to be an echolocator a priori on the basis of other evidence. The latter extrapolatory approach

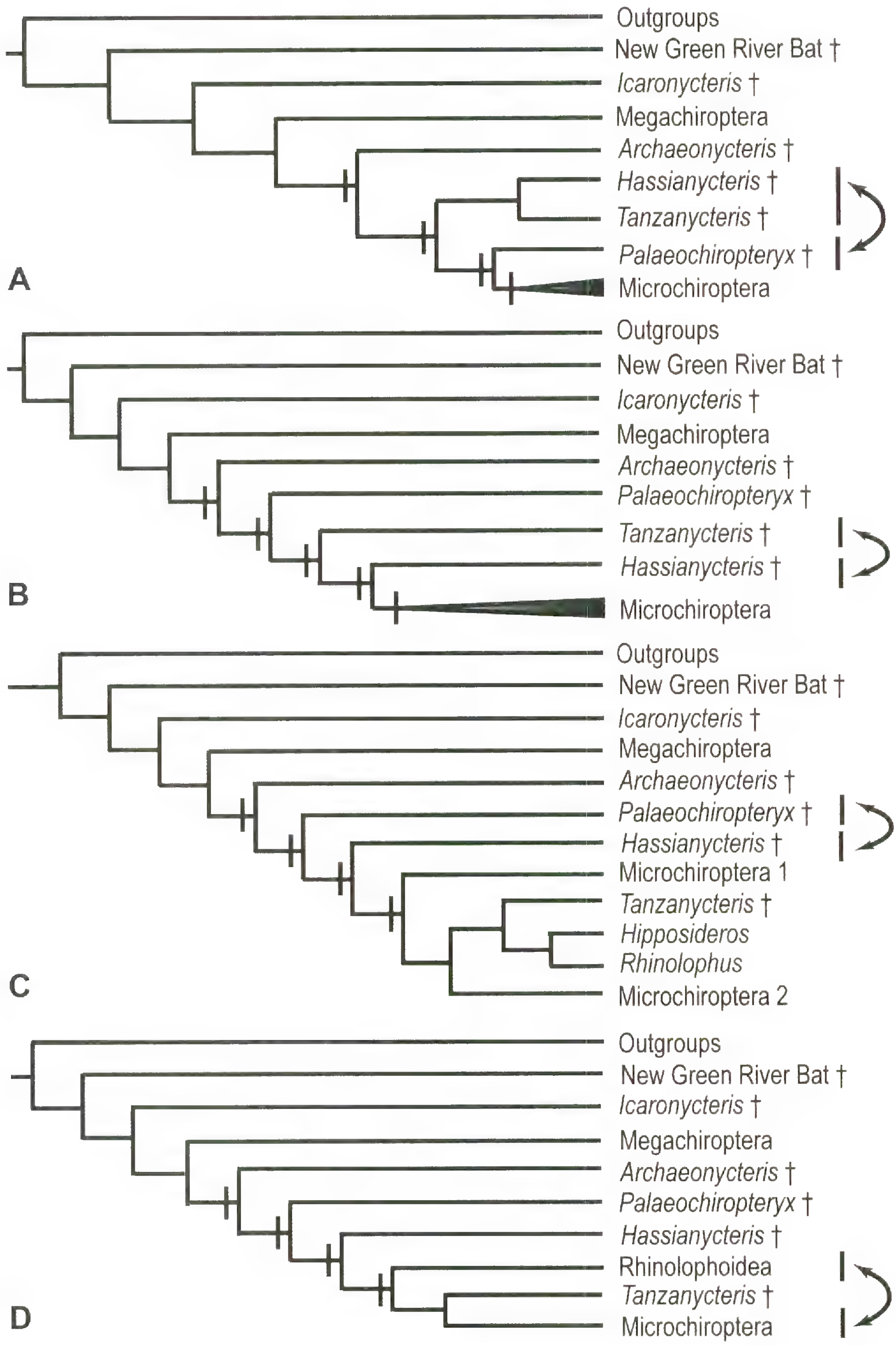

Figure 3. Four scenarios (each representing four MPTs) of character evolution based on 16 MPTs resulting from analysis of combined morphological and molecular sequence data sets without constraints (analysis 3b; see Fig. 1). In each case, arrows indicate a reversal of the positions of taxa in two of four MPTs. Fossil taxa are indicated by daggers (†). Equally optimal positions for a transition from absence to presence of laryngeal echolocation are indicated by hash marks. Each scenario supports one origination of laryngeal echolocation with no losses. Mapping of this character does not unambiguously affirm the hypothesis that *Archaeonycteris*, *Palaeochiropteryx*, and *Hassianycteris* were able to echolocate. *Tanzanycteris* is

(Bryant & Russell, 1992), of course, overrides the potential utility of phylogenetics to help one infer the presence or absence of behavioral characteristics that cannot be directly observed; furthermore, the phylogenetic topology and/or interpretation of character evolution may be impacted by choosing a character state on the basis of inferred rather than observed characteristics or behaviors. Mapping of co-varying structural characters that indicate the presence of a particular behavior may be one way to circumvent this apparent conundrum (e.g., Springer et al., 2001). However, when this reasoning is employed, it renders questionable the coding of both structural and behavioral characters in the matrix on which the analysis is based—as they are in the Chiropteran but not the Cetacean data set—since doing so would violate the assumption of character independence.

Another promising avenue for hypothesis testing is the potential utility of phylogenies to provide a framework on which the sequence and timing of character evolution can be juxtaposed; this is important, for instance, for corroborating or refuting proposed correlations between the evolution of structural features and extrinsic selective forces that occurred during geologic time. Hypothetical instances of this are given by Maddison and Maddison (2000) and Hermsen and Hendricks (2007). Both examples rely on the existence of a clade of extant taxa that is characterized by a unique adaptation that has been hypothesized to have arisen due to a selective force (e.g., appearance of a predator, climatic shifts, etc.). The extant clade is then analyzed with fossil taxa, which are found to nest within it. These taxa are older than the selective force that was hypothesized to have favored the adaptation that is a synapomorphy for the clade, so the cause-effect hypothesis is rejected.

As in determining minimum and maximum possible ages for the appearance of synapomorphies, the utility of this approach is biased—it is much easier to reject a cause-effect relationship between an extrinsic factor and the appearance of a synapomorphy if the minimum age of that synapomorphy is older than the selective pressure that supposedly favored its establishment. Clarke et al. (2007) provided a good example of this in penguins, although it is not directly linked to one specific character. Molecular divergence dating has suggested that the origin of the Spheniscidae (the clade including all extant penguins) occurred in the Paleogene (~40 Ma), possibly "concomitant with the initiation of the circum-Antarctic current, initial onset of Cenozoic global cooling, or at the proposed extinction of giant penguins" (Clarke et al., 2007: 11549). Clarke et al. (2007) cast doubt on these correlations through simultaneous analysis of fossil and extant penguins, which suggested instead that Speniscidae arose in the Neogene, based on the stratigraphic occurrences of the fossil penguins included in their analysis. They noted that accommodating a Paleogene–Spheniscidae radiation given their inferred phylogeny would require a 164.1–334.2 million year ghost lineage (estimated using MSM* [Manhattan Stratigraphic Measure], Pol & Norell, 2001). The extensive penguin fossil record known from between 40 Ma and 8 Ma includes no fossils that can be assigned to Spheniscidae. Despite this, they noted that their evidence is suggestive, not conclusive, since "stratigraphic data can only falsify a divergence date when a fossil discovery is older than estimated" (Clarke et al., 2007: 11549).

Conclusions

The challenge for biologists and paleontologists in coming years will be to more fully integrate our expanding knowledge of phylogeny as elucidated using molecular sequence data with our growing knowledge of the fossil record. Molecular sequence data have certainly helped to clarify relationships among extant taxa and will likely continue to modify and improve our understanding of the tree of life. Although fossil taxa almost always lack sequence data, they represent extinct morphologies that may be informative to the overall history of life on earth, as well as evolution within particular extant and extinct groups of organisms.

Several perceived obstacles to identifying the phylogenetic positions of fossil relative to extant taxa have been addressed above. Some of these purported problems are closely related to fossil taxa in particular (e.g., missing data), while others are related to morphological data in general (e.g., convergence and character weighting). These issues have been overemphasized as obstacles to effectively including fossil taxa in phylogenetic analyses, despite significant empirical advances in our understanding of how well the simultaneous analysis (or supermatrix) approach

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unambiguously inferred as possessing laryngeal echolocation in eight of 16 trees (C, D). In scenario C, Microchiroptera 2 includes the extant genera *Rhinopoma* E. Geoffroy, *Craseonycteris* Hill, *Megaderma* E. Geoffroy, and *Macroderma* Miller and Microchiroptera 1 contains all other ingroup microbat taxa. In scenario D, Rhinolophoidea includes *Rhinolophus* Lacépède, *Hipposideros* Gray, *Rhinopoma*, *Craseonycteris*, *Megaderma* and *Macroderma*, and Microchiroptera represents the remainder of the microbats.

often works for elucidating the phylogenetic context of fossil taxa relative to the extant biota. A priori arguments that fossil taxa should not be included in simultaneous analyses are insupportable—the suitability of fossil taxa for inclusion in these data sets needs to be tested empirically in the context of each data matrix and their behavior evaluated on a case-by-case basis (for instance, using pseudofossil analyses). The molecular scaffold approach has several weaknesses as a method for integrating morphological and molecular sequence data in order to include fossil taxa in phylogenetic hypotheses. Perhaps the largest of these is that the methodology obscures secondary signals that may manifest themselves during simultaneous analysis, that it minimizes the impact of fossil taxa on tree topology, and, when a semi-strict molecular scaffold is employed, it provides a contradictory perspective on the reliability of molecular sequence and morphological data.

A final note should be added in reference to paleobotany in particular, since the subject of this symposium is paleobotany in the post-genomics era. Most examples of simultaneous (and scaffold) analyses including fossil taxa in this paper come from studies of vertebrates (in addition to studies cited above, see, for instance, Brochu, 1997; Shaffer et al., 1997; O'Leary, 1999; Gao & Shubin, 2001; O'Leary et al., 2004; Asher et al., 2005b; Démére et al., 2005; Geisler & Uhen, 2005; Horovitz et al., 2006; Ksepka et al., 2006). This is because vertebrate paleontologists and zoologists have been leaders in the field, exploring methods to integrate fossil and extant taxa in greater numbers than those who study plants (in addition to the studies cited above, see Sun et al., 2002; Hermsen et al., 2003; Gandolfo et al., 2004; Crepet et al., 2005; Xiang et al., 2005) or invertebrates (in addition to the studies cited above, see Littlewood & Smith, 1995; Smith et al., 1995; Arango & Wheeler, 2007; Cardinal & Packer, 2007). Some of this may be the result of the large number of osteological characters that can be scored for vertebrates, perhaps making them more amenable to such analyses than other groups. Paleontologists and biologists who study vertebrates are also leading the way in the study of character evolution on phylogenies including extant and fossil taxa (see examples discussed above). While botanists have certainly kept pace in the arena of collecting sequence data and building phylogenies of extant organisms from those data, more work needs to be done to integrate our evolving view of the relationships of extant groups to one another with our knowledge of morphology, development, and the fossil record. Only integration of these disparate data types will provide us with a holistic view of plant evolution.

Addendum

Following the completion of the analyses documented in this paper, the "Green River bat" was formally published as *Onychonycteris finneyi* Simmons, Seymour, Habersetzer & Gunnell, 2008. Along with the formal description of this bat, Simmons et al. (2008) published new morphological and molecular scaffold phylogenetic analyses. These analyses were based on an updated morphological matrix and a molecular scaffold derived from a more recent molecular analysis than the Teeling et al. (2005) data used in our study. Notably, structural evidence from the newly published taxon is interpreted as confirming our inference that *Onychonycteris* was incapable of echolocation.

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THE WHOLE AND THE PARTS: RELATIONSHIPS BETWEEN FLORAL ARCHITECTURE AND FLORAL ORGAN SHAPE, AND THEIR REPERCUSSIONS ON THE INTERPRETATION OF FRAGMENTARY FLORAL FOSSILS[1]

Peter K. Endress[2]

ABSTRACT

Floral architecture and floral organ shape are interrelated to some extent as can be seen in the diversity of extant angiosperm groups. The shape of fragmentary fossil material, such as single organs, may therefore give hints for the reconstruction of the architecture of a flower. This study is partly a review and partly provides original material and new points of view on organ-architecture interrelationships. Several topics are illustrated with examples: (1) autonomous and imprinted shape, exemplified by cuneate organs, especially stamens; (2) conditions for valvate anther dehiscence; (3) lability in number and shape of reduced organs that have decreased in size and lost their original function; (4) long hairs as filling material of irregular spaces; (5) architectural conditions for the presence of orthotropous ovules; (6) structural differences between exposed and covered organ parts in bud; and (7) sepal aestivation and petal elaboration.

Key words: Autonomous shape, cuneate, floral architecture, imprinted shape, reduced organs.

There are relationships between the whole and the parts in floral structure. The ensemble of the floral organs constitutes the floral architecture, and, vice versa, the architecture of the whole flower or even inflorescence may have repercussions on the shape of individual organs. Under certain conditions, organ shape is conspicuously affected by the floral architecture. Thus, the shape of isolated organs may tell something about the architecture of flowers. Floral fossils are often not complete flowers, but are instead fragments and sometimes single organs. The question then arises on how the whole flower may have looked. Can certain traits of a flower be inferred from single organs, even if the systematic affinity is unknown? This is possible to some extent.

The influence of the floral architecture on the floral parts may be direct, shaped individually by neighboring organs during development. It may also be more indirect, shaped historically by evolutionary constraints on the best fit of the components of the floral architecture (Endress, 1975, 1994a). The first is seen in pressure marks on organs made by adjacent organs. The second is seen in harmoniously fitting parts within a (floral) bud. Both are related to economic use of space and protective function in the bud. In flowers at anthesis, the floral organs are also more or less highly synorganized with each other, which is related to the floral function in reproductive biology (Endress, 1994a, 2006).

The reconstruction and interpretation of floral fossils are important aspects in the integration of fossils into phylogenetic and evolutionary research. Information extracted from floral fossils is continuously being improved by technical advances in preparation and reconstruction (Schönenberger, 2005; De Vore et al., 2006; Friis et al., 2007; von Balthazar et al., 2007) and by the inclusion of fossils in morphological or combined molecular and morphological character matrices of extant plants for the determination of their phylogenetic position (Crepet & Nixon, 1998; Gandolfo et al., 1998; Sun et al., 2002; Friis et al., 2003a; Hermsen et al., 2003, 2006; Crepet et al., 2004). Conversely, information from extant flowers helps in the interpretation of related floral

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fossils (Crane et al., 1989; Friis & Endress, 1990; Endress & Friis, 1994, 2006; Doyle et al., 2003; Hermsen et al., 2003; Eklund et al., 2004).

This study tries to shed light on a neglected phenomenon that is informative in the understanding of floral architecture and organ shape, for paleobotanical reconstruction, and would also be of interest from a molecular developmental and evolutionary perspective. It is partly a review, but also presents original material.

Material and Methods

Species and specimens used for original illustrations are listed in Appendix 1.

Material used for light microscopy (LM) or scanning electron microscopy (SEM) was fixed in formalin/acetic acid/alcohol (FAA) or 70% ethanol. For serial microtome sections, specimens were either embedded in paraplast and the section series stained with safranin and Astra blue or embedded in Technovite (2-hydroethyl methacrylate; Hereus Kulzer, Wehrheim, Germany) and stained with ruthenium red and toluidine blue. Sections were mostly 5–10 μm thick. For SEM studies, specimens were treated with 2% osmium tetroxide, dehydrated in ethanol and acetone series, critical point–dried, and sputter-coated with gold.

Autonomous Shape and Imprinted Shape (Superimposed by Contiguous Neighbors)

The influence of mechanical pressure on the shape of plant organs has rarely been studied, perhaps in contrast to animals (Benjamin & Hillen, 2003), although the influence of mechanical stimulation on plants is conspicuous (Braam, 2005). It is useful to distinguish between autonomous and imprinted shape. Autonomous shape is the shape of an organ developing without physical influence from contiguous organs. Imprinted shape is the shape of an organ altered by the pressure of contiguous organs (Endress, 1975, 1994a, 2006). There are various intensities of this effect. In the extreme case (level 1), irregular imprints of adjacent contiguous organs are present on the affected organ. This goes so far that the disassembled organs can be put together like the pieces of a jigsaw puzzle (Fig. 1A, B). If two such organs are of the same kind (e.g., two stamens), the mutual effect is about equal in both. If they are of different kinds, one of them is more deformed than the other. As an example, in a floral bud of an Annonaceae, the deforming influence of the stamens on the carpels is more pronounced than that of the carpels on the stamens for most of their length. This is shown on transverse sections by broad indentations within carpel flanks but lesser indentations in stamen flanks (Fig. 1A, left side). In addition, the peripheral stamens of the bud are shaped by the three inner perianth organs; the peripheral line is relatively smooth and shows three corners at the site of the margins of the three inner perianth organs (Fig. 1B). Thus, in these two examples it appears that the outer (older) organs influence the inner (younger) organs more than the other way round. In a less extreme case (level 2), there is no or less direct local deformation, but the organs fit tightly together in a harmonious way. As an example, in a floral bud of *Gillbeea adenopetala* F. Muell. (Cunoniaceae), the stamens of the two whorls have filaments of different lengths, and thus the anthers are orderly stacked in two superposed rows (Fig. 2A). However, there are also cases in which, in contrast to an orderly stacking, the floral organs are not orderly stuffed in bud, e.g., in flowers with a high number of stamens; such flowers may then have somewhat distorted anthers in bud, but the shape may be more or less restored when the flowers are open and the pressure is released, e.g., *Barringtonia calyptrata* R. Br. ex Benth., Lecythidaceae (Fig. 2B, C) (Endress, 1994a).

In general, it appears that stamens or carpels affected by imprinted shapes are more common in basal angiosperms and basal eudicots than in core eudicots or monocots. However, there are exceptions of such stamens in core eudicots, especially in flowers in which the perianth and androecium are not highly synorganized and that have irregular numbers of perianth and androecium organs. An example is the elusive genus *Sphenostemon* Baill., which was once classified (as *Idenburgia* Gibbs) with Trimeniaceae (Austrobaileyales) (Perkins, 1925), one of the basalmost angiosperm families. In complete contrast, today *Sphenostemon* is in a family of its own (Sphenostemonaceae) of uncertain position in the euasterids II (Savolainen et al., 2000; APG, 2003). Its earlier erroneous placement was because of the general appearance of its flowers and in part because of its irregularly cuneate stamens without an anther that is clearly differentiated from the filament. The stamens are densely crowded in bud and have an imprinted shape by mutual pressure (Fig. 2D–G) (see also Endress, 2002).

However, the reverse may also occur: imprinted shapes may play a role in highly regular and synorganized flowers. In Asclepiadoideae (Apocynaceae), the two carpels fuse postgenitally in their uppermost part and this united region attains a pentangular shape, molded by the five adjacent anthers (e.g., Endress, 1990). Thus, the apex of the anthetic gynoecium looks like a pentamerous struc-

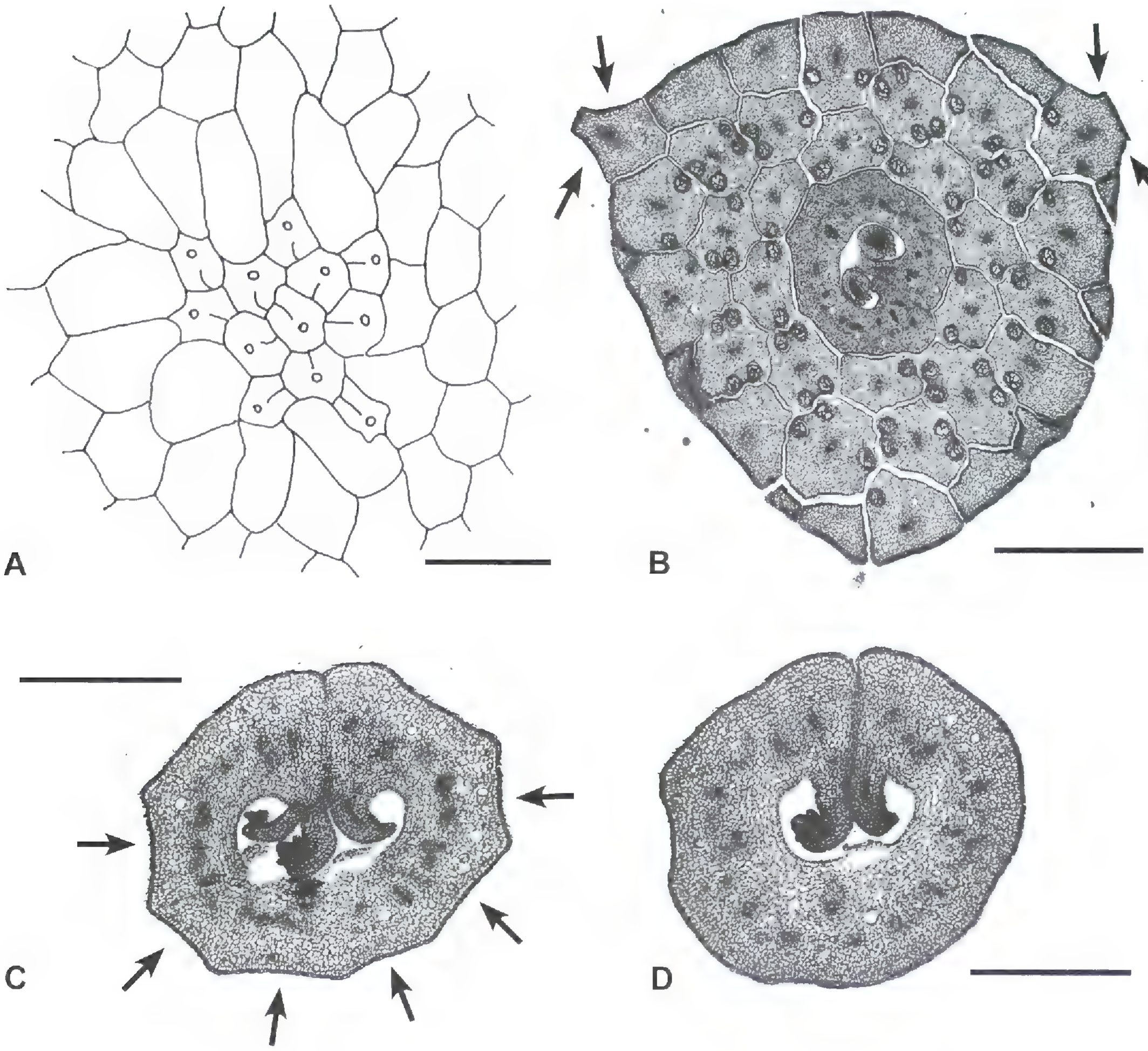


Figure 1. Autonomous and imprinted shape in flowers of Annonaceae. —A. *Cananga odorata* Hook. f. & Thomson, transverse section of floral center with inner stamens and carpels (with ventral slit and dorsal vascular bundle indicated). All organs are contiguous and arranged like the pieces of a jigsaw puzzle by mutually imprinted shapes. The section is slightly oblique (lower on the right side than on the left). On the left side, the stamens shape the carpels, and on the right side the carpels shape the stamens. B–D. *Cyathocalyx martabanicus* Hook. f. & Thomson, transverse sections. —B. Androecium and unicarpellate gynoecium. The stamens shape each other and the gynoecium, and the periphery of the androecium is shaped by the three inner (removed) tepals (arrows). —C. Gynoecium shaped by stamens (arrows). —D. Same gynoecium with autonomous shape at the base where the inner stamens are united into a ring and the imprinting influence of single stamens is lacking. Magnification bars: A, B = 1 mm; C, D = 0.5 mm.

ture, although it is dimerous. Mutual adjustment of adjacent organ surfaces is necessary for postgenital fusion of the five stamens with the upper part of the gynoecium in these highly synorganized flowers. The developmental processes involved in this adjustment are largely unknown (see also Endress, 2006).

However, such imprinted shapes are not commonly used for subsequent postgenital fusion. A similar quadrangular shape of a dimerous gynoecium, imprinted by four stamens, is present in *Triaenophora* Solereder (Plantaginaceae) (Wang & Wang, 2005). In some Lauraceae, the monomerous gynoecium attains a triangular shape in early development, molded by the trimerous preceding whorls. This has led to the erroneous interpretation of a trimerous nature of the lauraceous gynoecium (Singh & Singh, 1985), although monomery is evident from comparative developmental studies (Endress, 1972).

Shape of a floral primordium may also be influenced by adjacent parts of the inflorescence. Instead of being circular (*Couroupita* Aubl., Lecythidaceae; Fig. 3A), it can be transversely extended, especially if the inflorescence is elongate and the flowers have a reduced perianth (e.g., *Euptelea* Siebold & Zucc., Eupteleaceae, Fig. 3B, and Endress, 1986; Ren et al., 2007; *Styloceras* Kunth ex A. Juss., Buxaceae, von Balthazar & Endress, 2002). It can also be asymmetrical, if the inflorescence is monochasial

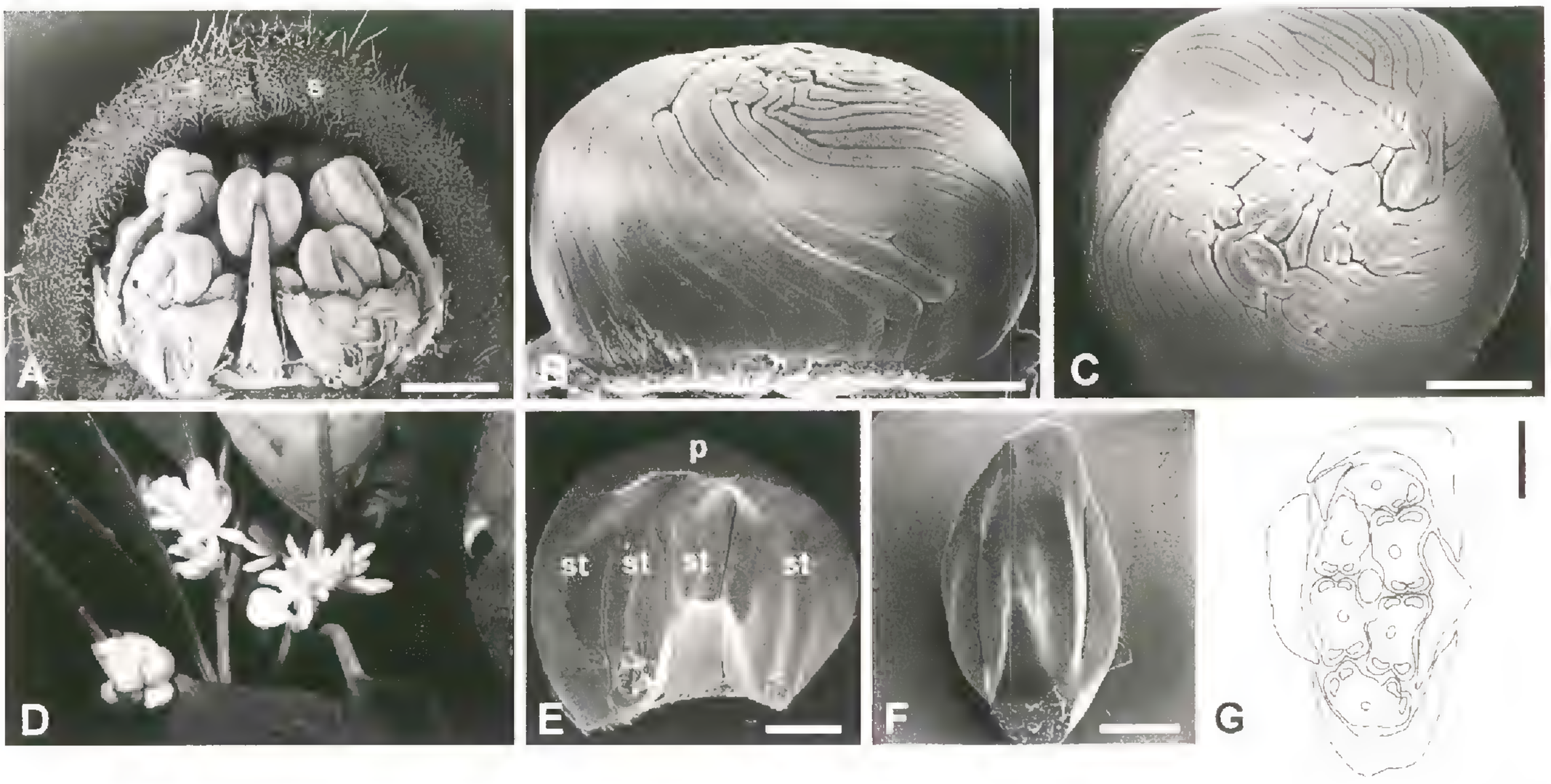


Figure 2. Spacing in buds. —A. *Gillbeea adenopetala* F. Muell. (Cunoniaceae). Regular spacing of the two whorls of five stamens within the floral bud, covered by five valvate sepals (s). B, C. *Barringtonia calyptrata* (Lecythidaceae), androecium, perianth removed. Irregular spacing (stuffing) of the numerous stamens in bud, anthers with imprinted shape by mutual deformation. —B. Bud view from the side. —C. Bud view from above. D–G. *Sphenostemon lobosporus* (F. Muell.) L. S. Sm. (Sphenostemonaceae). —D. Flowering shoot. —E. Perianth part (p) and four stamens (st) from bud, irregularly contiguous. —F. Stamen from bud (from ventral), showing irregular, imprinted shape. —G. Transverse section of bud with tight arrangement and imprinted shape of anthers. Magnification bars: A = 0.5 mm; B, C = 1 mm; E–G = 0.2 mm.

(e.g., Heliconiaceae, Strelitziaceae, Kirchoff, 2003). The difference in shape of floral primordia may be especially striking if inflorescences have both a lateral and a terminal flower, such as in *Drimys* J. R. Forst. & G. Forst. (Winteraceae), in which the different shape of the floral primordium has repercussions on floral phyllotaxis of lateral versus terminal flowers (Doust, 2001). Not only can floral primordia be asymmetric but inflorescence primordia can be as well, if they are under space constraints or under the influence of a polarity induced by the entire plant or a larger unit of the plant, such as in Loteae (Leguminosae) with a superposed dorsiventrality (Sokoloff et al., 2007). In *Euptelea* (see above), in addition to the

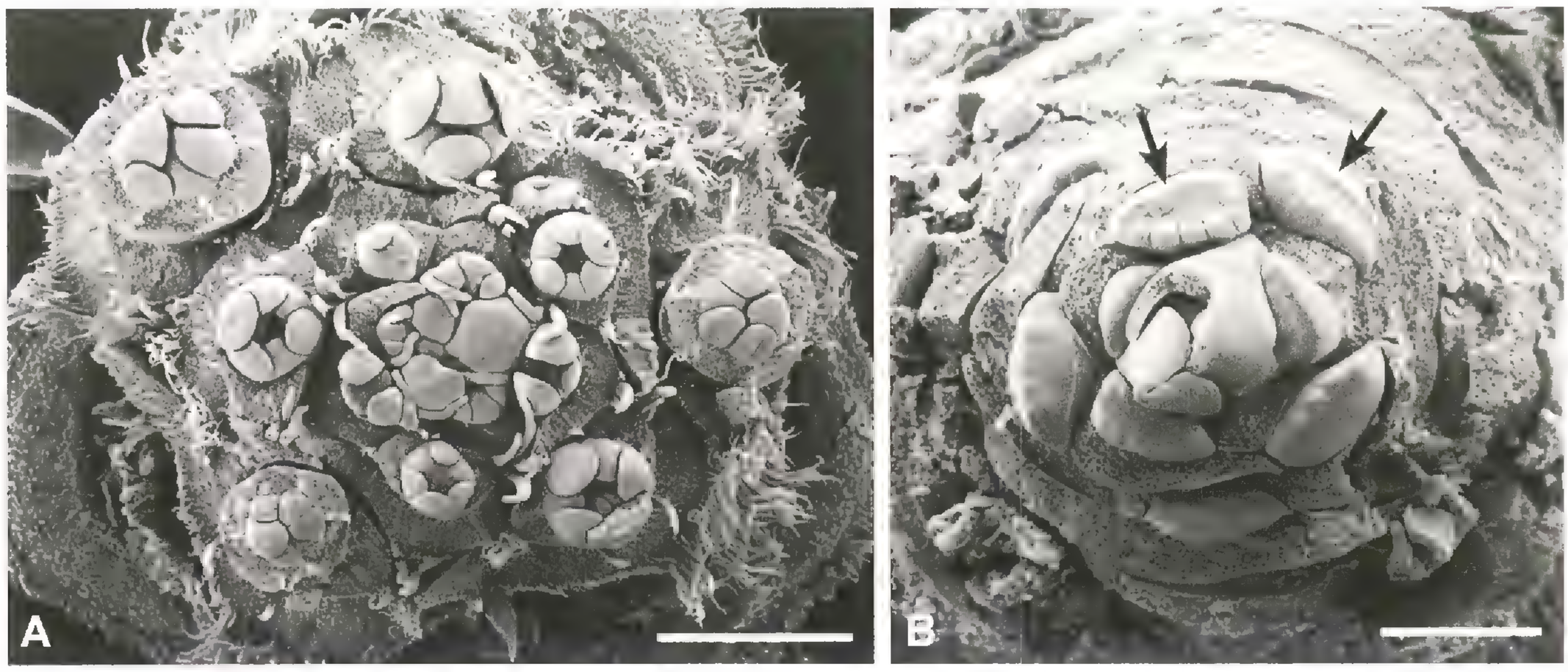


Figure 3. Shape of floral primordia. A–B. Young inflorescence tips from above. —A. *Couroupita guianensis* Aubl. (Lecythidaceae), floral primordia and young flowers widely spaced, round, with autonomous shape. —B. *Euptelea polyandra* Siebold & Zucc. (Eupteleaceae), floral primordia and young flowers crowded, flattened, with imprinted shape; the abaxial transverse line is caused by pressure of the subtending bract (arrows). Magnification bars: A = 1 mm; B = 0.5 mm.

general transversely extended shape, direct pressure by the subtending bract leaves its mark on the abaxial side of the young flower (Fig. 3B).

Architectural Conditions for the Presence of Cuneate or Shield-shaped Organs

An example for imprinted shape are Lower Cretaceous isolated fossil stamens, many of which show two striking features: (1) cuneate (wedge-shaped) anthers and a massive apex, and (2) anther dehiscence by laterally hinged valves and not by simple longitudinal slits (Friis et al., 1991, 2006a). Such cuneate stamens (anthers) also occur in some extant plants (Figs. 4, 5) (Endress, 1975; Endress & Hufford, 1989; Hufford & Endress, 1989). We may ask: Are cuneate stamens typical for certain clades, or is this shape due to certain architectural constraints independent of systematic relationship? Both questions can be answered affirmatively to some extent. The first aspect, cuneate shape, which is related to imprinted shape, will be addressed in this section; the second aspect, valvate dehiscence, will be addressed in the next section.

Isolated cuneate (wedge-shaped) anthers with a massive sterile apex and short filament indicate with some probability that the anthers were contiguous and densely arranged in bud, that the anther apex had a protective function and was not covered in bud by other organs, and that stamen arrangement was in a spherical, hemispherical, or cylindrical fashion. Therefore, the surface of the entire architecture is largest at the periphery, which results in the cuneate form of the stamens. There are two possibilities for such an architecture: (1) inflorescences with dense flower arrangement and the perianth lacking or small and not protective, such as in extant *Hedyosmum* Sw. (basal angiosperms) (Fig. 4A–C), Platanaceae (basal eudicots) (Fig. 4D–F), Altingiaceae (basal core eudicots) (Fig. 4G–I), Sparganiaceae (Fig. 4J–L), and Typhaceae (Fig. 4M–O) (both commelinid monocots), and (2) single flowers with numerous stamens, such as in Annonaceae (Fig. 5) and *Nuphar* Sm. in Nymphaeaceae, both basal angiosperms (Endress, 1975, 1987). In Platanaceae, the female inflorescences have a similar architecture as the male ones, and the carpels have a cuneate ovary (Crane, 1989). In some Annonaceae (although they have a well-developed protective perianth), the stamens become exposed in late bud, and thus their broad apices are then the protective parts for the thecae and the ovaries. In both cases, the massive anther apices may also be a protection against pollinators with biting mouth parts or other herbivorous insects. Thus, for isolated fossil stamens with such a shape, even if they may not be determined as to their larger alliance (if pollen is lacking), partial reconstruction of the architecture of their flowers or inflorescences may be possible.

Cuneate carpels arranged in a spherical gynoecium occur in *Kadsura marmorata* (Hend. & Andr. Hend.) A. C. Sm. in Schisandraceae (Smith, 1947), and cuneate gynoecia (without perianths and subtending bracts) arranged in a spherical inflorescence occur in *Pandanus* S. Parkinson (pers. obs.).

There are not only cuneate floral organs but also other kinds of cuneate organs with a protective function and similar dense arrangement into cone-like architectures. In extant plants, bracts that subtend flowers or partial inflorescences (cymes) are often cuneate or have a shield-like portion at the periphery of the bud of dense inflorescences (e.g., Piperaceae [Fig. 6A–C], Mimosoideae of Leguminosae [Fig. 6D–F], and Betulaceae [Fig. 6G–I], Endress, 1975; Balanophoraceae, Kuijt, 1969; Moraceae, Berg, 1990). This is also true for sporangiophores of various gymnosperms and groups other than seed plants (e.g., *Equisetum* L. [Fig. 6P–R], Endress, 1975; Taxaceae [Fig. 6M–O], Endress, 1975; Mundry & Mundry, 2001; cycads [Fig. 6J–L], Endress, 1975; Mundry & Stützel, 2003). Cuneate organs are also known from a number of various fossil plants. Subtending bracts (or sporangiophores) are cuneate in some lycophytes (*Mazocarpon* Benson; Schopf, 1941; Pigg, 1983), sphenophytes (*Palaeostachya* Weiss; Baxter, 1955), cycads (*Androstrobus* Schimper, *Beania* Carruthers, Harris, 1941, 1964; *Delemaya* Klavins, Taylor, Krings & Taylor, Klavins et al., 2003), and many conifers (Ohsawa, 1994; Stockey, 1994). Ovules are cuneate in Pentoxylales (Sahni, 1948; Crane, 1985) and interseminal scales in Bennettitales (Harris, 1969; Crepet, 1974; Sharma, 1982; Pedersen et al., 1989; Nishida, 1994; Rothwell & Stockey, 2002; Stockey & Rothwell, 2003). The formation of such massive peripheral, contiguous protective parts is commonly provided not by a small-celled, cytoplasm-rich marginal meristem but mainly by cell enlargement (Endress, 1975). The well-preserved Bennettitales described by Crepet and Delevoryas (1972) also indicate that cell enlargement alone was instrumental in the thickening of the peripheral, protective part of the ovular integument in female reproductive structures.

Conditions for Valvate and Simple Longitudinal Dehiscence in Anthers

The second striking feature of fossil Lower Cretaceous stamens (Friis et al., 2006a: fig. 21), in addition to the cuneate shape (see above), is that a number of them have a valvate dehiscence pattern with two laterally hinged valves in each of the two

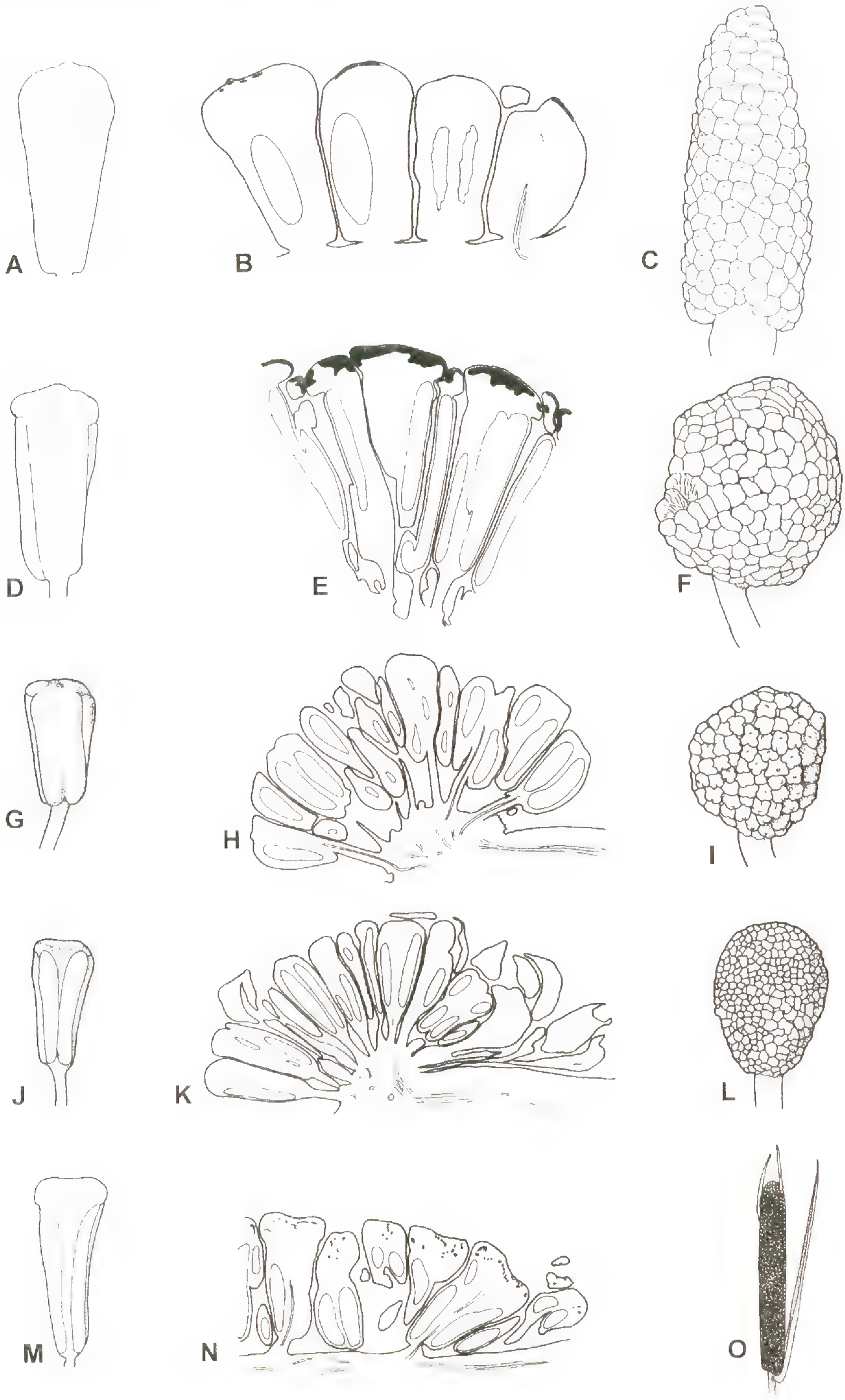


Figure 4. Cuneate anthers and spherical or cylindrical young inflorescences bearing flowers with reduced perianth. —A, D, G, J, M. Stamen (anther). —B, E, H, K, N. Longitudinal section of part of the inflorescence showing contiguous stamens. —C, F, I, L, O. Entire inflorescence. A–C. *Hedyosmum mexicanum* C. Cordem. (Chloranthaceae). D–F. *Platanus orientalis* L. (Platanaceae). G–I. *Liquidambar styraciflua* L. (Altingiaceae). J–L. *Sparganium erectum* L. (Sparganiaceae). M–O. *Typha minima* Funck in Hoppe (Typhaceae). Tanniferous tissue black in B, E, N. (From Endress, 1975, with permission; <http://www.schweizerbart.de>).

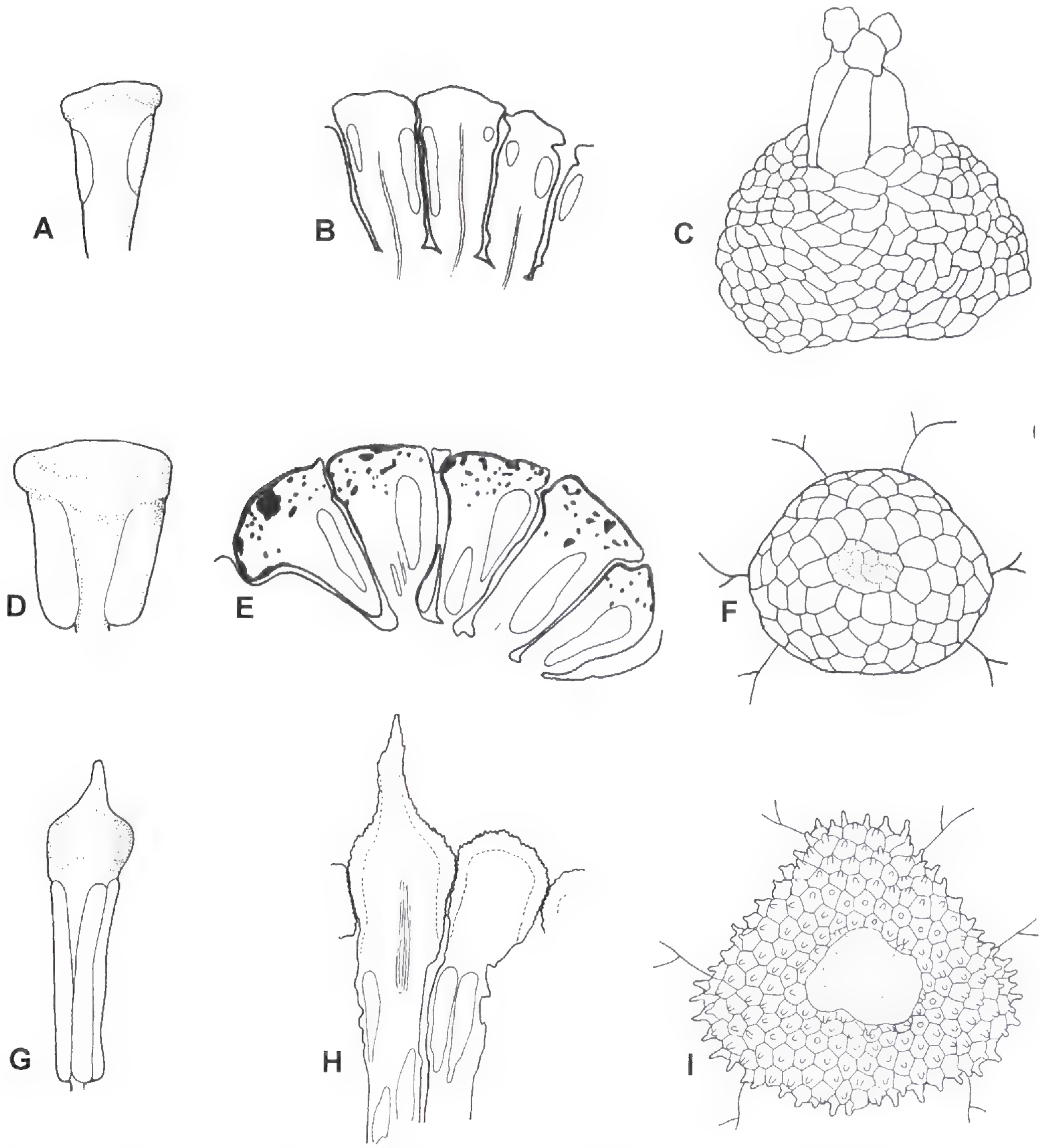


Figure 5. Cuneate anthers and hemispherical androecia in Annonaceae. —A, D, G. Stamen (anther). —B, E, H. Longitudinal section of part of the androecium showing contiguous stamens. —C, F, I. Androecium and gynoecium. A–C. *Asimina triloba* (L.) Dunal. D–F. *Polyalthia longifolia* (Sonn.) Thwaites. G–I. *Cananga odorata* (Lam.) Hook. f. & Thomson. Tanniferous tissue black in E. (From Endress, 1975, with permission; <http://www.schweizerbart.de>).

thecae, which open in the manner of saloon doors; this is also true for extant taxa with a similar anther shape. A variant of laterally hinged valves are apically hinged valves, which do not act in pairs that open together but open each pollen sac separately (restricted to some Laurales). Anthers with a thin connective (and therefore bulging pollen sacs) can easily be deformed after dehiscence (Fig. 7A–C), which allows efficient opening, and for such opening it is sufficient to have simple longitudinal dehiscence. Anthers with a thick connective, however, cannot be deformed so easily because of architectural constraints, and, therefore, valvate dehiscence is more efficient (Fig. 7D–F).

Valvate stamens with this architecture (thick connective) are concentrated in some clades and not common in angiosperms as a whole. Although these clades are in disparate places in the angiosperm tree, they are more or less restricted to basal angiosperms and basal eudicots. Such anthers were recorded among basalmost angiosperms in Nymphaeales (*Nuphar*; Hufford, 1996); among other basal angiosperms in Laurales (*Sinocalycanthus* (Cheng & S. Y. Chang) Cheng & S. Y. Chang and some fossil Calycanthaceae;

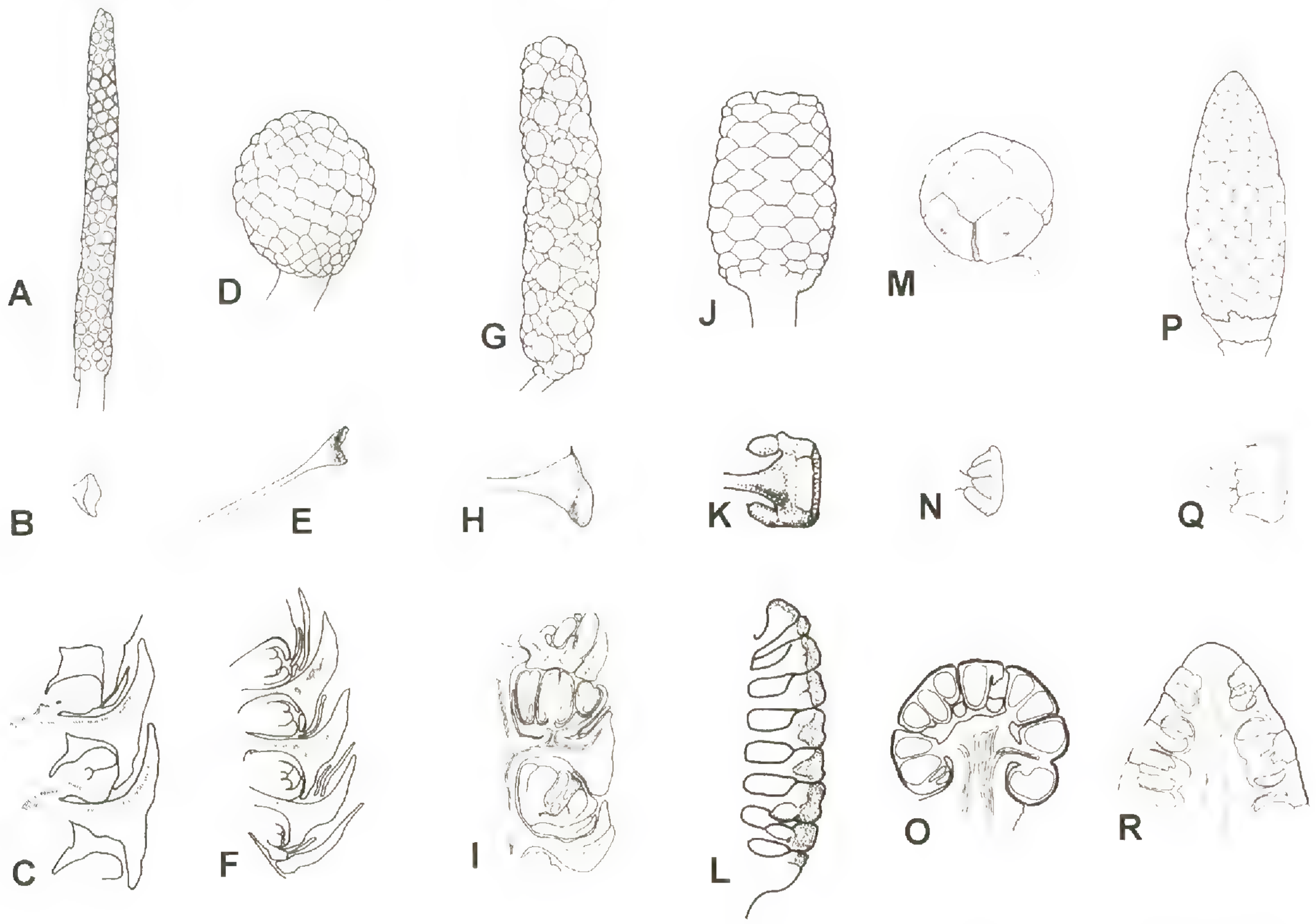


Figure 6. Cuneate subtending bracts of flowers or partial inflorescences or sporangiophores, and hemispherical or cylindrical inflorescences or shoots with sporangiophores. —A, D, G, J, M, P. Inflorescences or shoots with sporangiophores. —B, E, H. Subtending bracts. —K, N, Q. Sporangiophores. —C, F, I, L, O, R. Longitudinal sections of (parts of) A, D, G, J, M, and P. A, B. *Peperomia rotundifolia* (L.) Kunth (Piperaceae). C. *P. caperata* Yunck. (Piperaceae). D–F. *Leucaena latisiliqua* (L.) Gillis & Stearn (Leguminosae). G–I. *Alnus glutinosa* (L.) Gaertn. (Betulaceae). J–L. *Zamia verschaffeltii* Miq., female (Zamiaceae). M–O. *Taxus baccata* L. (Taxaceae). P–R. *Equisetum arvense* L. (Equisetaceae). (From Endress, 1975, with permission; <http://www.schweizerbart.de>).

Friis et al., 1994; Crepet et al., 2005; Staedler et al., 2007; Atherospermataceae, Lauraceae, Hernandiaceae, Monimioideae–Monimiaceae; Endress & Hufford, 1989), Magnoliales (Annonaceae [Fig. 8A], Magnoliaceae, Eupomatiaceae, Himantandraceae, Degeneriaceae; Endress & Hufford, 1989), and Piperales (*Piper augustum* Rudge; Endress, 1994b); among basal eudicots in Ranunculales (Eupteleaceae; Endress, 1986; Ranunculaceae p.p.; Endress & Hufford, 1989; Weber, 1993), Proteales (Platanaceae [Fig. 8B]; Hufford & Endress, 1989), and Trochodendraceae (Endress, 1986; Hufford & Endress, 1989; Chen et al., 2007); and among basal core eudicots in Saxifragales (Altingiaceae, Hamamelidaceae [Fig. 8C]; Endress, 1989; Hufford & Endress, 1989). Valvate anthers appear to be absent from monocots and higher core eudicots.

Lability in Number and Shape of Reduced Organs That Have Decreased in Size and Lost Their Original Function

Reduced organs that have decreased in size (i.e., have become shorter and narrower) and lost their original function may become labile in number and shape because functional constraints on shape are lacking. A reduction in size and loss of function of an organ type may be concomitant with a decrease in number, but the reverse—an irregular increase in number—is also possible. What are the conditions for maintaining a constant number of organs in whorls?

In eudicots, sepals are basically the protective organs in floral buds. They are commonly quincuncial-imbricate (with overlapping flanks) or, less often, valvate (with margins of adjacent sepals tightly appressed to each other). Especially in the latter case, all sepals of a flower have the same shape and their number is stable, as they have to build a precise envelope for the young floral organs (e.g., *Heisteria* Jacq., Olacaceae) (Fig. 9A). However, here and there in the phylogenetic tree, the protective function has been evolutionarily transferred from the sepals to another organ category (e.g., petals) or, less often, floral subtending bracts or prophylls. If petals have become protective, they commonly become larger than the sepals early in development and often have a valvate aestivation in bud. The sepals have then

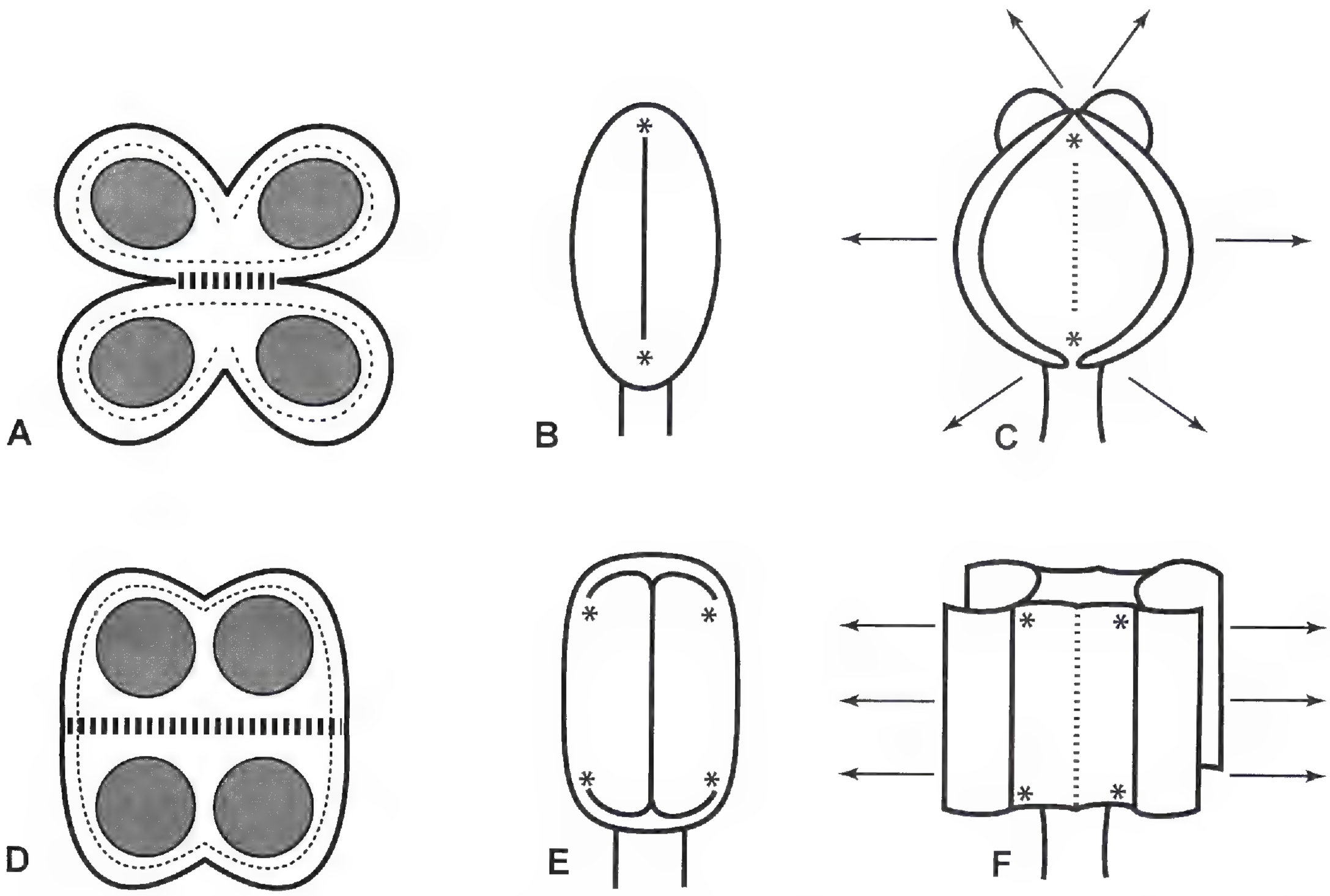


Figure 7. Anthers with opening by longitudinal slits (A–C) and laterally hinged valves (D–F). —A, D. Transverse section of anther before dehiscence (thick, interrupted line indicates thickness of anther; thin, interrupted lines indicate endothecium; gray areas indicate pollen sacs). —B, E. Anther before dehiscence, from transverse side, showing theca with dehiscence lines (asterisks indicate ends of dehiscence lines). —C, F. Anther after dehiscence, from transversal side (interrupted line indicates septum between the two pollen sacs of a theca; asterisks indicate ends of dehiscence lines; arrows indicate directions of opening of theca).

commonly become small and sometimes irregular in shape and number. In some cases, the number has irregularly increased. This irregularity is due to a lack of a functional constraint on a precise number and shape of the organs. An example is *Mimosa* L. (Leguminosae), in which several subgroups have a valvate corolla (Fig. 9C) and a highly reduced calyx, which is irregularly denticulate, with more teeth than

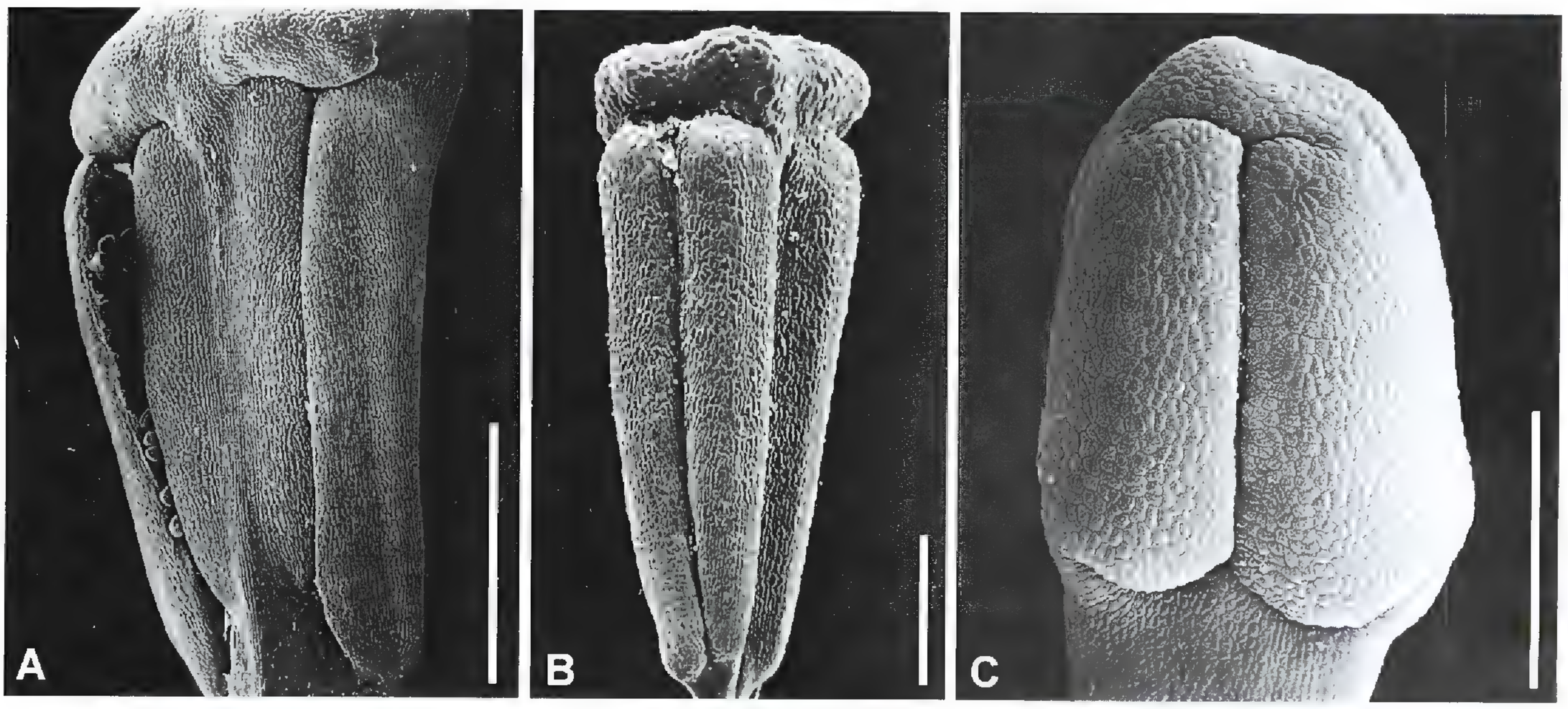


Figure 8. Anthers with dehiscence by two laterally hinged valves for each theca. —A. *Artabotrys hexapetalus* (L. f.) Bhandari (Annonaceae). —B. *Platanus orientalis* L. (Platanaceae). —C. *Fothergilla major* Lodd. (Hamamelidaceae). Magnification bars: A–C = 0.5 mm.

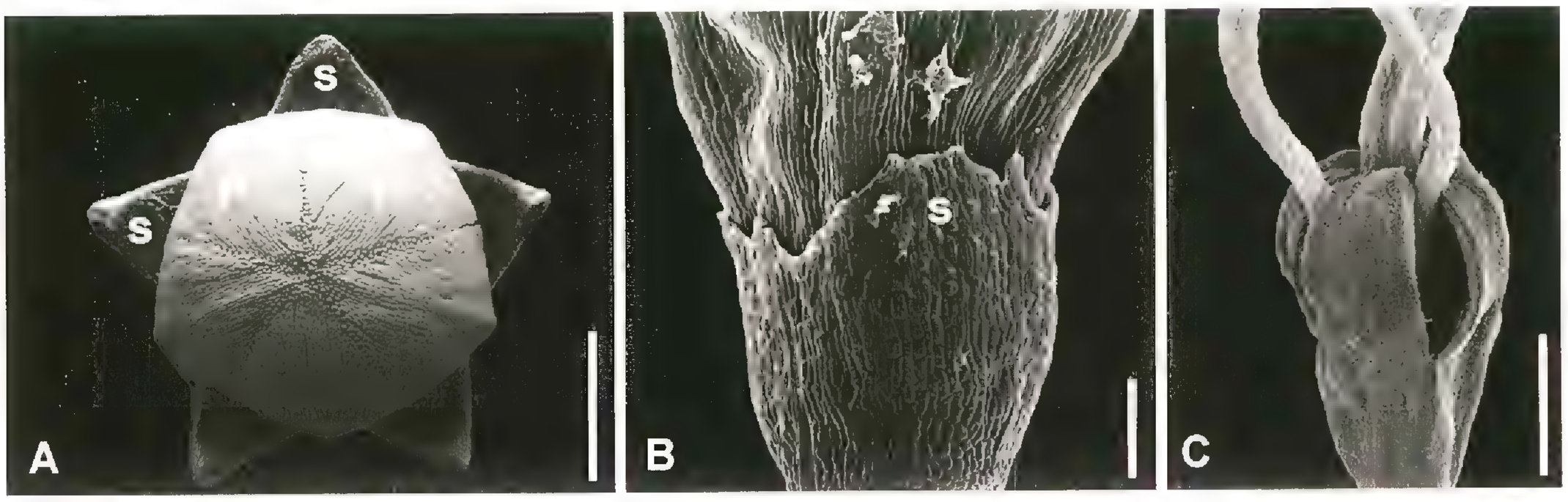


Figure 9. Well-developed versus reduced perianth organs. —A. *Heisteria parvifolia* Sm. (Olacaceae), sepals and petals well developed, both with valvate aestivation. B, C. *Mimosa spegazzinii* Pirotta (Leguminosae). —B. Sepals reduced, with open aestivation. —C. Petals well developed, with valvate aestivation. p = petals, s = sepals. Magnification bars: A, C = 0.5 mm; B = 0.1 mm.

would be expected from the merism of the flower (Fig. 9B) (see also Tucker, 1984; Barneby, 1991). Even more conspicuous examples are Asteraceae and Valerianaceae, in which the originally pentamerous calyx is dissolved into more than five pappus bristles (e.g., Semple, 2006).

Thunbergia Retz. and related genera (Thunbergioideae, Acanthaceae) (Schönenberger & Endress, 1998; Schönenberger, 1999) are examples for bracts as protective floral organs. As in most Lamiales, the basic sepal number in Acanthaceae is five, and the sepals are protective in bud. However, in Thunbergioideae, the mechanical protective function for the flowers has been transferred to two large prophylls, which are postgenitally united in bud (Fig. 10A). In contrast, the sepals are not protective at any stage of floral development (except indirectly for bearing extranuptial nectaries, which attract ants that defend the flowers against herbivores) (Fig. 10B). Thus, the sepals are much reduced in size and sometimes completely lost. However, if present, they often have irregularly increased in number from originally five to 15 or more in some species; they form short scales of slightly variable size. In sphingid-pollinated species (e.g., *T. guerkeana* Lindau) with increased flower length, these ca. 15 narrow organs may be secondarily elongate in terms of evolution (Fig. 10C) (Schönenberger, 1999).

Subtending bracts are another possibility for surrogate protective organs if the sepals are reduced. In Balanophoraceae, which has massive protective bracts, male flowers of *Helosis* Rich. still have

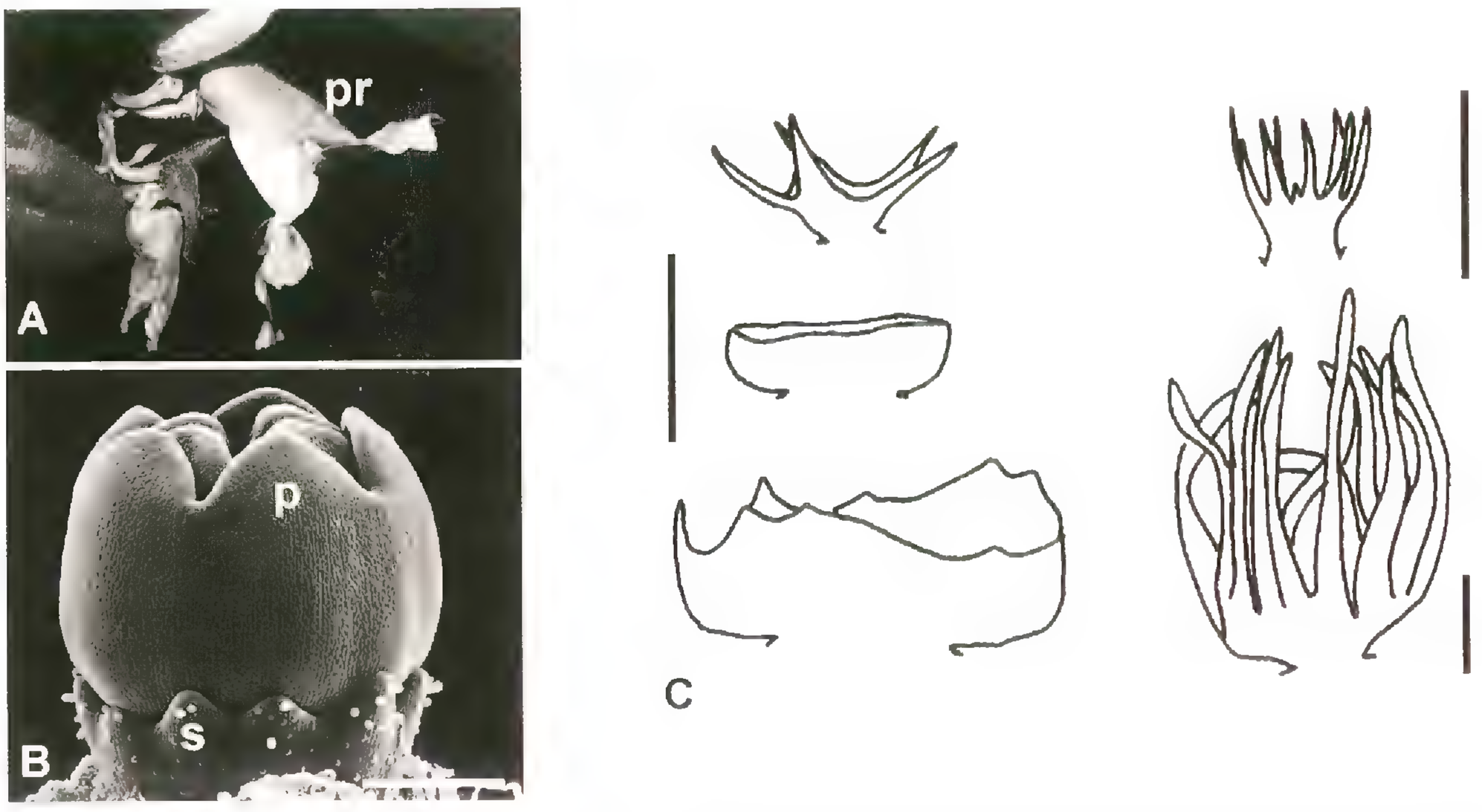


Figure 10. *Thunbergia*, reduced calyx. —A. *T. grandiflora* Roxb., open flower from the side. —B. *T. alata* Sims, young flower bud. —C. Calyx differentiation in five species of *Thunbergia*. p = petal, pr = prophyll, s = sepal. Magnification bar: B = 0.25 mm; C = 5 mm.

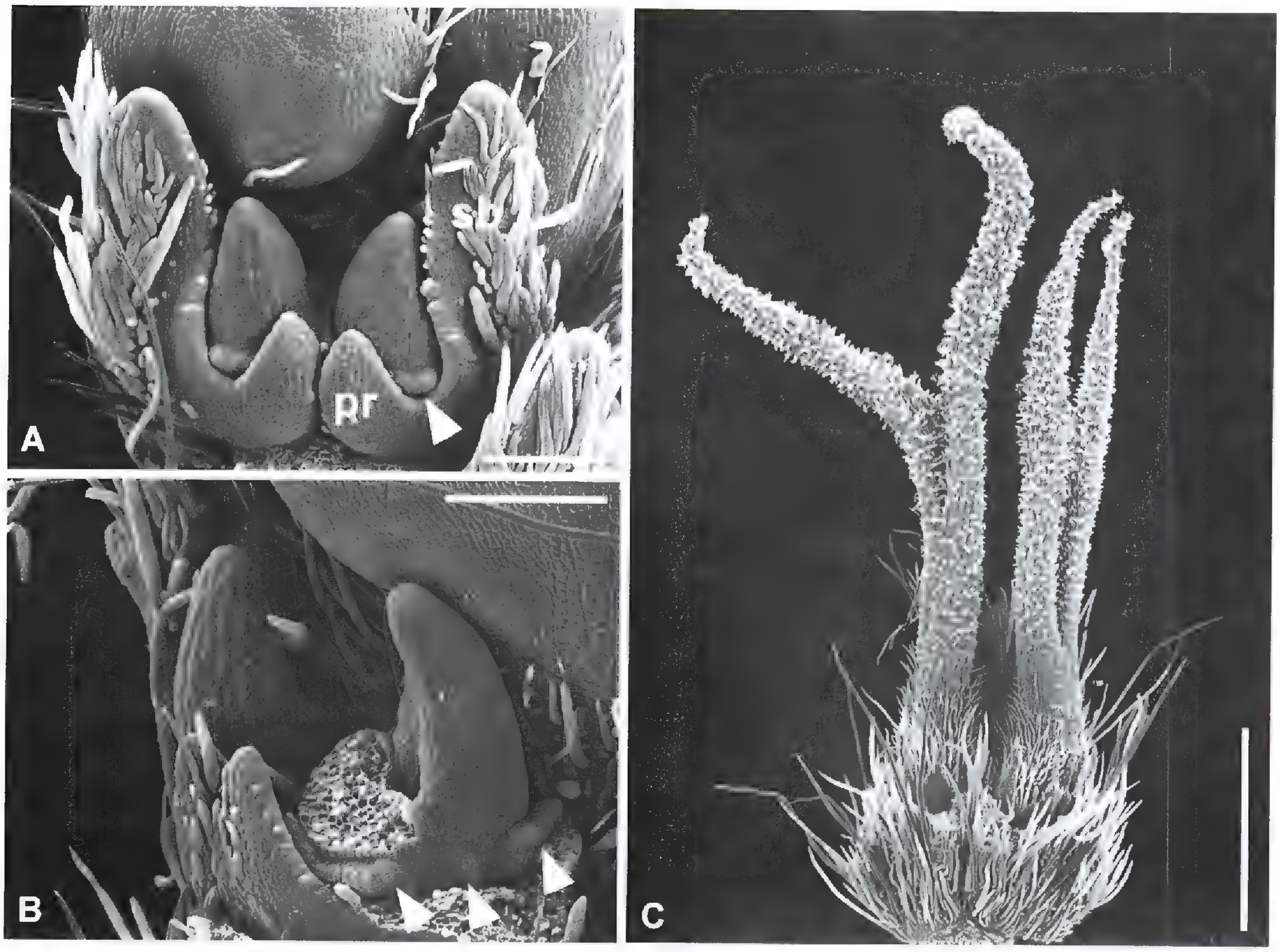
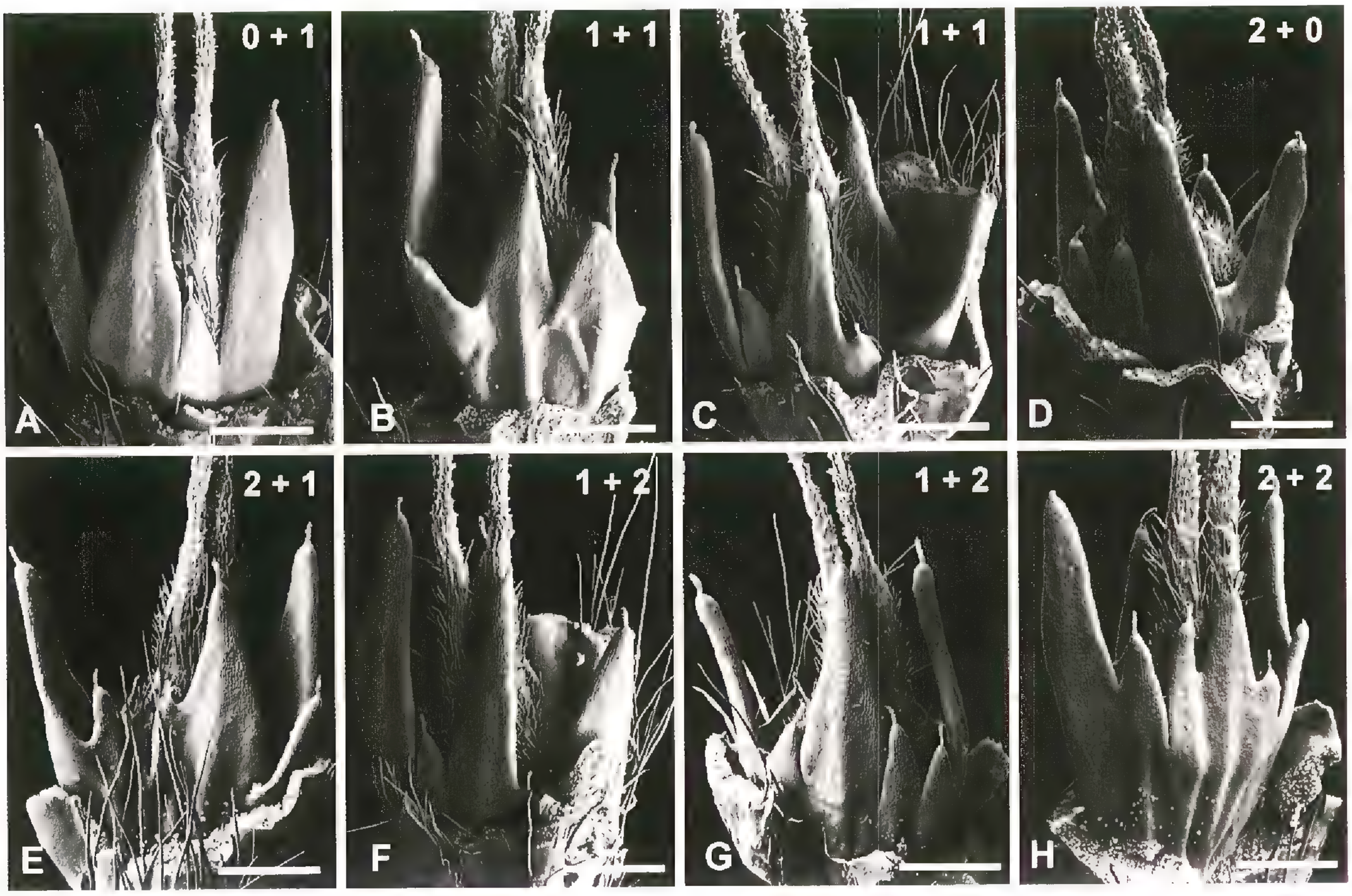


Figure 11. *Carpinus betulus* (Betulaceae), female flowers, with reduced calyx. —A, B. Two flower buds in a dichasium at sepal initiation; in B, one of the carpels of one of the two flowers removed. —C. Two flowers in a dichasium, at anthesis. pr = prophyll, sb = subtending bract, arrowheads = sepal primordia. Magnification bars: A, B = 0.2 mm; C = 1 mm.

protective, valvate sepals of a fixed number, whereas those of the closely related *Corynaea* Hook. f. have nonprotective, reduced sepals with irregular shape and increased, unstable number (Kuijt, 1969). *Parrotia* C. A. Mey. and *Sycopsis* Oliv. (Hamamelidaceae) also have reduced, nonprotective sepals with irregular shape and increased, unstable number, in contrast to other Hamamelidaceae with larger, protective sepals with a fixed number and shape (Endress, 1989, 1990).

A last example, female flowers of *Carpinus* L. (Betulaceae), should be treated in more detail. In contrast to the male flowers of *Alnus* Mill. and *Betula* L. with four and two protective sepals, according to tetramerous and dimerous flowers, respectively, in *Carpinus* (Abbe, 1935), the sepals are not protective; they are short, of irregular shape, and increased in number. However, although in *C. betulus* L. sepal number and shape are irregular (Eichler, 1878), there are commonly four somewhat larger sepals in the median and transverse planes, which is still reminiscent of a basic tetramerous pattern (Abbe, 1935; Endress, 1967). Protective organs for the young flowers are the subtending bract of each flower pair, which represents a dichasium without a central flower, plus the subtending bract and the two prophylls of each single flower (Fig. 11C). The sepals are much delayed in development, and at no time do they enclose the inner parts of the flower. Sepal delay is so pronounced that they only appear after the two carpels have been initiated. They first appear as two irregular rims, each in the median plane of the bicarpellate gynoecium (Fig. 11A, B). Each sepal (or calyx tooth) ends in a colleter in early development—a secretory tip that probably has a chemical, and not mechanical, protective function for the young flower (Figs. 12, 13). Later in development, pronounced longitudinal ribs develop in the calyx teeth extending along the surface of the inferior ovary and are most conspicuous in fruit (Fig. 13). In these ribs, strong vascular bundles are situated, which probably differentiate so strongly due to the early secretory function of the colleters, which they serve. Such pronounced ribs are also present in fossil Betulaceae-like flowers (Schönenberger et al., 2001; Friis et al., 2003b, 2006b).

In *Carpinus betulus*, structural lability is expressed at the level of the individual (Fig. 12A–H) and between individuals (Fig. 13A–F). Within an individ-



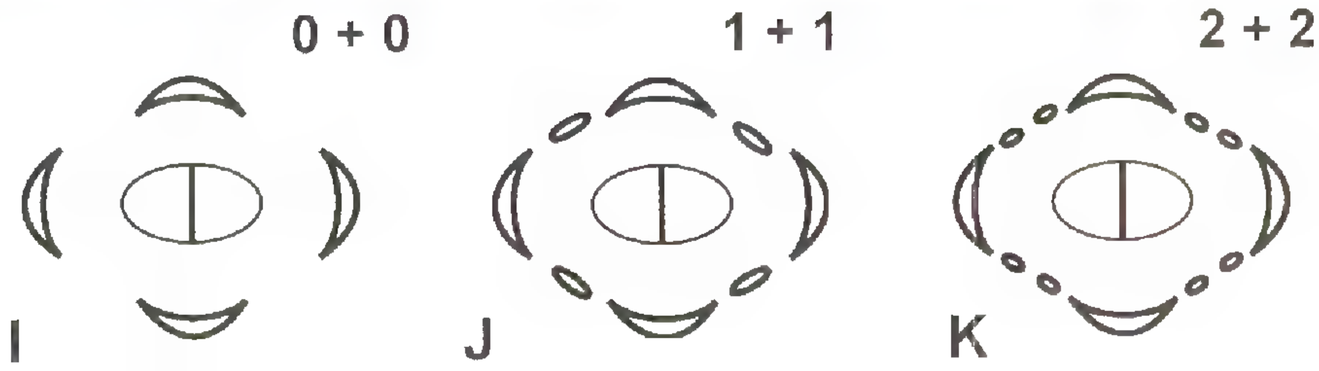


Figure 12. *Carpinus betulus* (Betulaceae). —A–H. Female flowers from a single tree showing intra-individual variability of sepal formation, with corresponding sepal formula of the visible side indicated at upper right. — I–K. Floral diagrams with corresponding sepal formula of one side of the flower indicated. Magnification bars: A–H = 0.5 mm.

ual, between the commonly four larger sepals in the median and transverse planes there may be zero, one, or two commonly smaller sepals. Within a flower, all these numbers may be present without a recognizable pattern (Fig. 12A–K). Inter-individual variation can be seen by comparing developmental series of flowers from two different trees (Fig. 13A–F). In the second tree, the sepals are shorter, more numerous, and, in older stages, more curved so that in the fruit they form a more convoluted rim (Fig. 13F) than in the first tree (Fig. 13C).

Thus, in both *Thunbergia* (Acanthaceae) and *Carpinus* (Betulaceae), the sepals appear to have convergently lost their mechanical protective function with size reduction and concomitant loss of a fixed size, shape, and number, but perhaps secondarily gained a secretory protective function. Therefore, small sepals of irregular size associated with secretory structures in fossil flowers may indicate that they did not have a mechanical protective function and that their number may be variable.

Increase in number concomitant with reduction in size and loss of function is not only present in sepals. It may also occur in stamens. An example is the large genus *Bauhinia* L. (Leguminosae), in which the basic number of 10 stamens in two whorls as common in the family may be reduced to five, three, or even a single stamen. In *B. galpinii* N. E. Br., only the three lower (anterior) stamens of the outer androecial whorl are well developed (Fig. 14A, D). The other seven stamens are variously reduced to small staminodes without anthers (Fig. 14C, D) (Endress, 1994a). The two upper staminodes of the outer whorl are still larger than those of the inner whorl. In the inner whorl, the organs are increased in number. In the expected position of each single organ, there are two or three tiny collateral organs (Fig. 14B, D). In *Dillenia* L. (Dilleniaceae), the flowers have numerous stamens

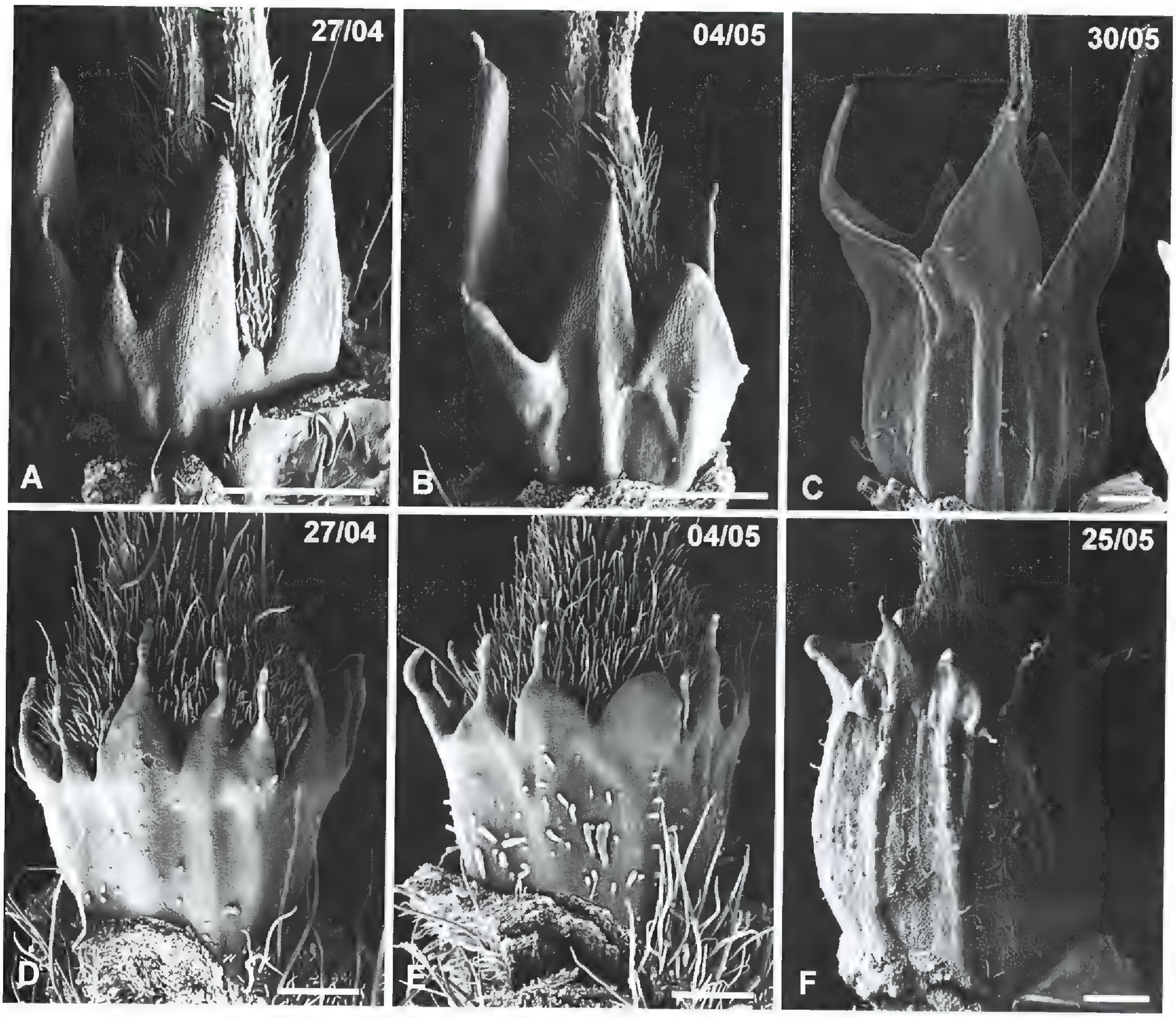


Figure 13. *Carpinus betulus* (Betulaceae). A–F. Female flowers from two different trees, in the same developmental stages (dates of collection indicated), showing inter-individual variability of sepal formation. —A–C. Same tree as Figure 12A–H. —D–F. Another tree. Magnification bars: A–C, F = 0.5 mm; D, E = 0.25 mm.

that are centrifugally initiated. The last initiated organs of the androecium are staminodes. They are narrower than the stamens, and two collateral organs occupy a position in which a single organ would be expected (Endress, 1997: fig. 8B).

Long Hairs as Filling Material of (Irregular) Spaces

The presence of long hairs concentrated in certain areas of flowers is sometimes an indication of irregular spaces in flower buds, as hairs may be used as flexible filling material, which may have protective functions against herbivores or physical factors (e.g., drought or frost). Such hairs occur preferably at or around carpel bases. There they may also play a role in fruit development. Examples are seen in Hamamelidaceae and Cunoniaceae (Matthews & Endress, 2002), which have only two carpels but have five, 10, or more stamens. In these flowers, long hairs are at and around the ovary (Fig. 15A–D). In some Monimiaceae, the carpels are free in the bottom of a floral cup, and hairs develop in the interspaces between the carpels (Fig. 15E) (Endress, 1980). In *Munroidendron* Sherff (Araliaceae), the hairs are filling material for the open space in the floral center that originates by an increased number of carpels in a whorl (Fig. 15F) (Endress, 2006). In Moraceae, protective hairs between floral organs or flowers are also common (Berg, 1990).

Another instance is hairs on anthers, as seen in male flowers of *Carpinus* (and other Coryleae, Betulaceae). Here, sepals are not only reduced as in female flowers but completely absent. The inner space of the bud is provided by the subtending floral bract and the bracts of the adjacent flowers, and has an irregular shape so that the anthers cannot be tightly packed in a strict pattern. Thus, the space is not spherical, as often in flowers, but broad and flat. To fill this space, these flowers have two unusual features: (1) the two thecae of each anther are

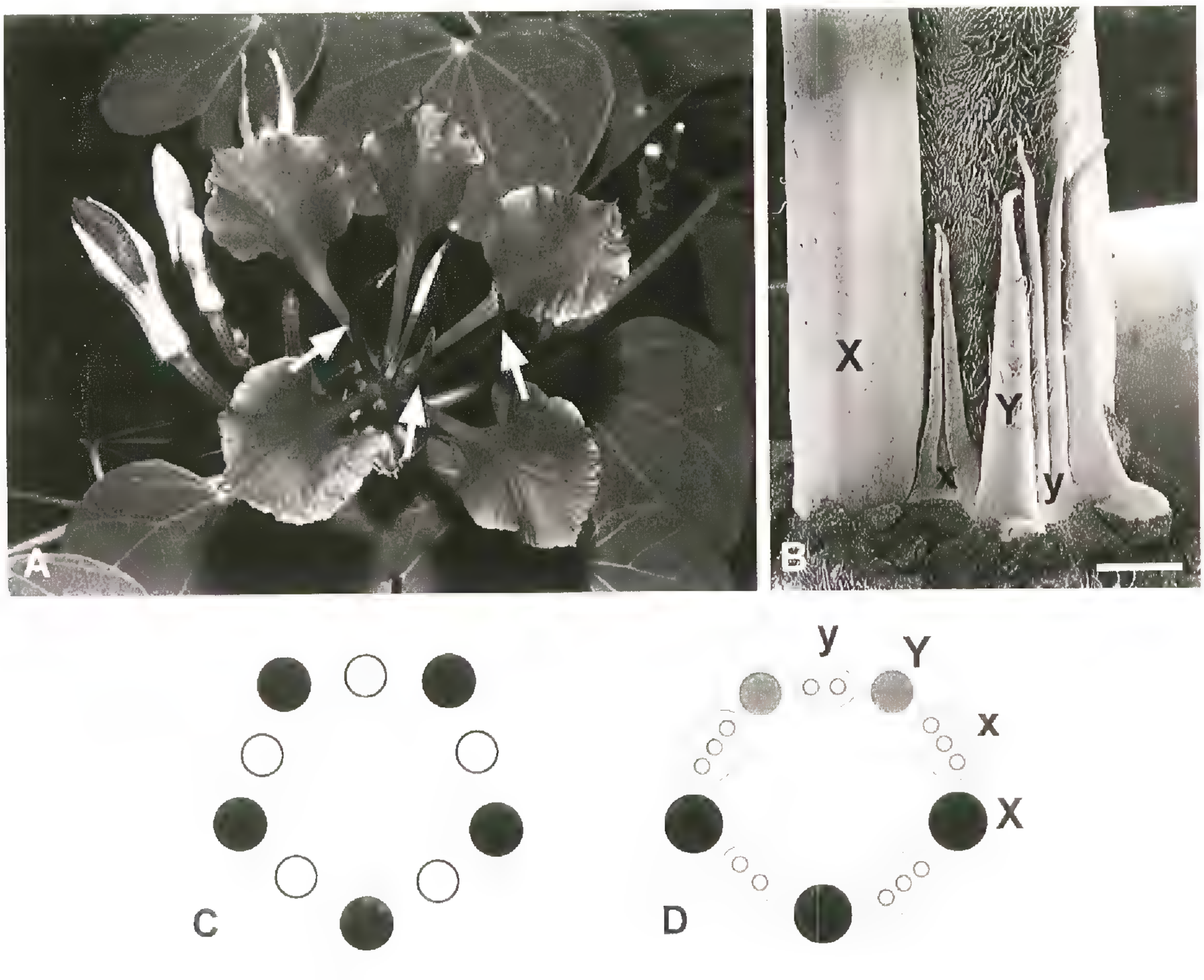


Figure 14. *Bauhinia galpinii* N. E. Br. (Leguminosae), reduction of androecium and concomitant increase in number of reduced organs. —A. Flower with three fertile stamens (arrows). —B. Stamen and staminode of outer whorl (X, Y) and staminodes increased in number of inner whorl (x, y). —C. Diagram of androecium common in many Leguminosae (black: organs of outer whorl, white: organs of inner whorl). —D. Diagram of androecium of *Bauhinia galpinii*, showing increase in number of strongly reduced staminodes of inner whorl (black and gray: organs of outer whorl; white: organs of inner whorl; letters corresponding to those in Fig. 14B). Magnification bar: B = 0.5 mm.

separated from each other by a filamentous connective, which allows flexible arrangement of the thecae (Fig. 15H); and (2) again, long hairs fill empty spaces. These hairs occur as tufts on top of each theca (Fig. 15G, H). Such hairs on anthers are present in a few unrelated groups of wind-pollinated plants with a reduced perianth (e.g., some Anacardiaceae, Fagaceae, Juglandaceae) (Elias, 1972; Endress & Stumpf, 1991; Bachelier & Endress, 2007). It would also be interesting to know whether the presence of hairs on these anthers is just a consequence of sepal reduction and a concomitant irregular space in bud, or whether it has acquired an additional ecological function in the context of pollen dispersal by wind.

Architectural Conditions for the Presence of Orthotropous Ovules in Angiosperms

In contrast to other seed plants, angiosperms commonly have anatropous ovules. Ovules with this shape have the micropyle topographically adjacent to the placenta and, thus, can take up pollen tubes directly from the placenta. Nevertheless, orthotropous ovules occur sporadically or regularly in some larger groups. They conspicuously tend to occur under certain conditions of the ovary architecture, three of which are listed here. Commonly, the inner architecture of the ovary is such that the pathway of pollen tubes from the stylar canal to the micropyle of the ovules is alleviated by the orthotropous shape.

(1) The most simple condition is the presence of a single basal (or almost basal) ovule, which positions its micropyle directly to the lower end of the stylar canal, thus enabling direct passage of a pollen tube (Fig. 16A, D, G). This condition has evolved in many different angiosperm families, such as in Piperaceae (basal angiosperms; Igersheim & Endress, 1998), Didymelaceae (basal eudicots; von Balthazar et al., 2003), Polygonaceae p.p. (core eudicots, Caryophyllales), Juglandaceae and Myricaceae (core eudicots, rosids, Fagales), Urticaceae (core eudicots, rosids, Rosales; Eckardt, 1937), Zosteraceae (basal monocots; Dahlgren, 1939), and Araceae (basal monocots).

(2) If the locule is filled with secretion, ovules of any number can be orthotropous as the pollen tubes may directly grow through the secretion (e.g., Buzgo, 1994) (Fig. 16B, E, H). This condition is known from a number of plants that grow in wet or moist habitats,

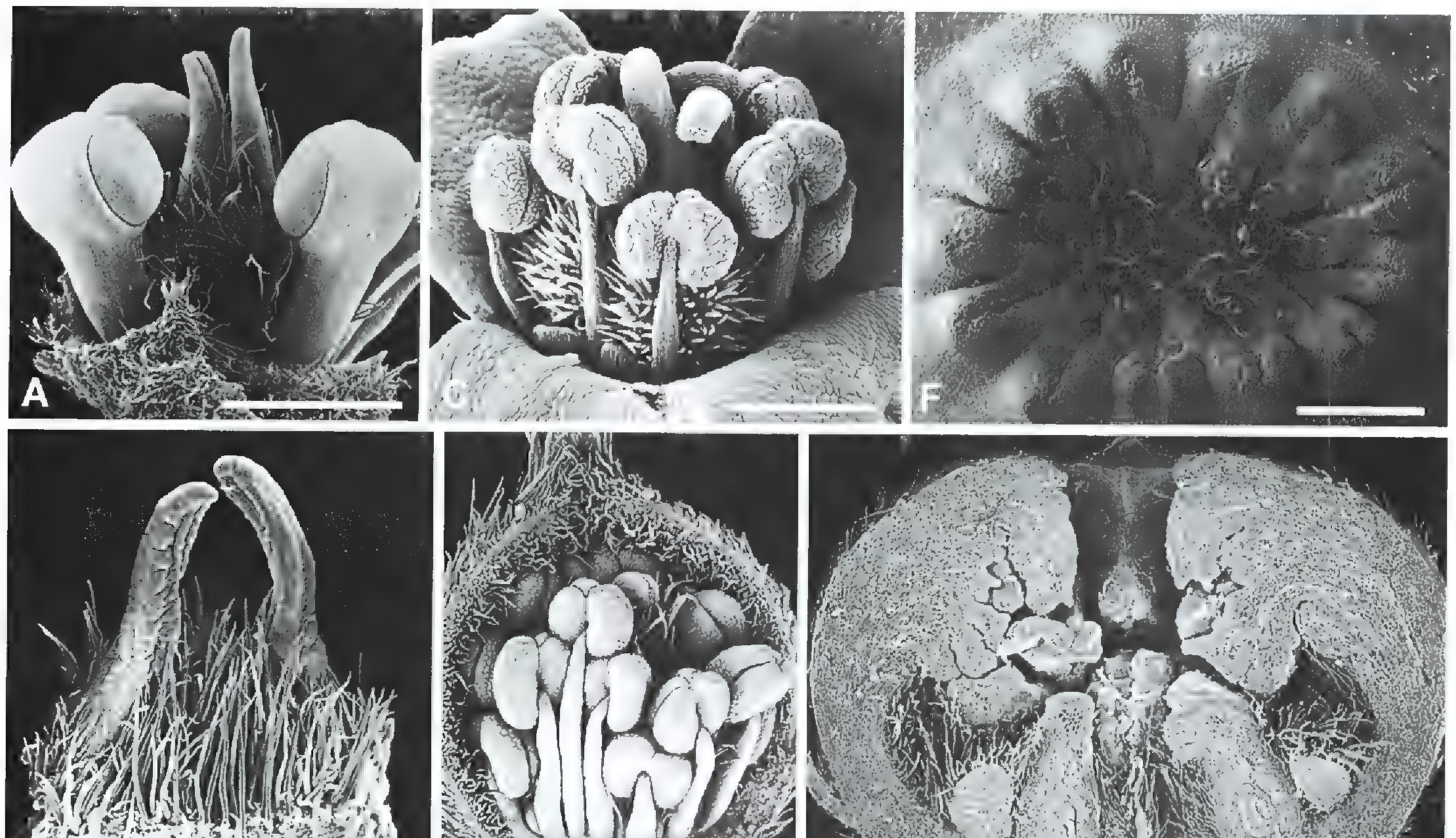


Figure 15. Long hairs as filling material of empty spaces in floral buds and flowers. —A. *Hamamelis virginiana* L. (Hamamelidaceae). —B. *Trichocladus grandiflorus* Oliv. (Hamamelidaceae). —C. *Acsmithia davidsonii* (F. Muell.) Hoogland (Cunoniaceae). —D. *Geissois biagiana* F. Muell. (Cunoniaceae). —E. *Wilkiea* sp. indet. (Monimiaceae). —F. *Munroidendron racemosum* (C. N. Forbes) Sherff (Araliaceae). G, H. *Carpinus betulus*, male (Betulaceae). —G. Part of male inflorescence, flower-subtending bracts partly removed. —H. Stamen, with the two thecae flexible by filamentous connective and crowned by tuft of long hairs. Magnification bars: A, B, F = 0.5 mm; C = 0.3 mm; D = 0.4 mm; E, G = 1 mm; H = 2 mm.

such as in *Barclaya* Wall. of Nymphaeaceae (basalmost angiosperms; Schneider, 1978; Igersheim & Endress, 1998), Acoraceae (basal monocots; Rudall & Furness, 1997; Buzgo & Endress, 2000; Igersheim et al., 2001), *Pistia* L. and other Araceae (basal monocots; Buzgo, 1994), and some Hydrocharitaceae (basal monocots; Igersheim et al., 2001).

(3) If the placentae are parietal, the micropyle of orthotropous ovules may be directed not to their own placenta but to the neighboring placenta, where they take up pollen tubes (Fig. 16C, F, I). Examples are *Casearia* Jacq. (core eudicots, rosids, Malpighiales) and Mayacaceae (monocots, commelinids).

These three architectural conditions for the presence of orthotropous ovules were briefly discussed in Endress (1994a). There are also other instances of orthotropous ovules, in which the ovary architecture does not so evidently favor the orthotropous condition as in the three types mentioned.

Structural Differences Between Exposed and Covered Organ Parts in Bud

Organ surfaces that are exposed in bud may behave differently from those that are covered in bud. This is especially conspicuous in imbricate aestivation,

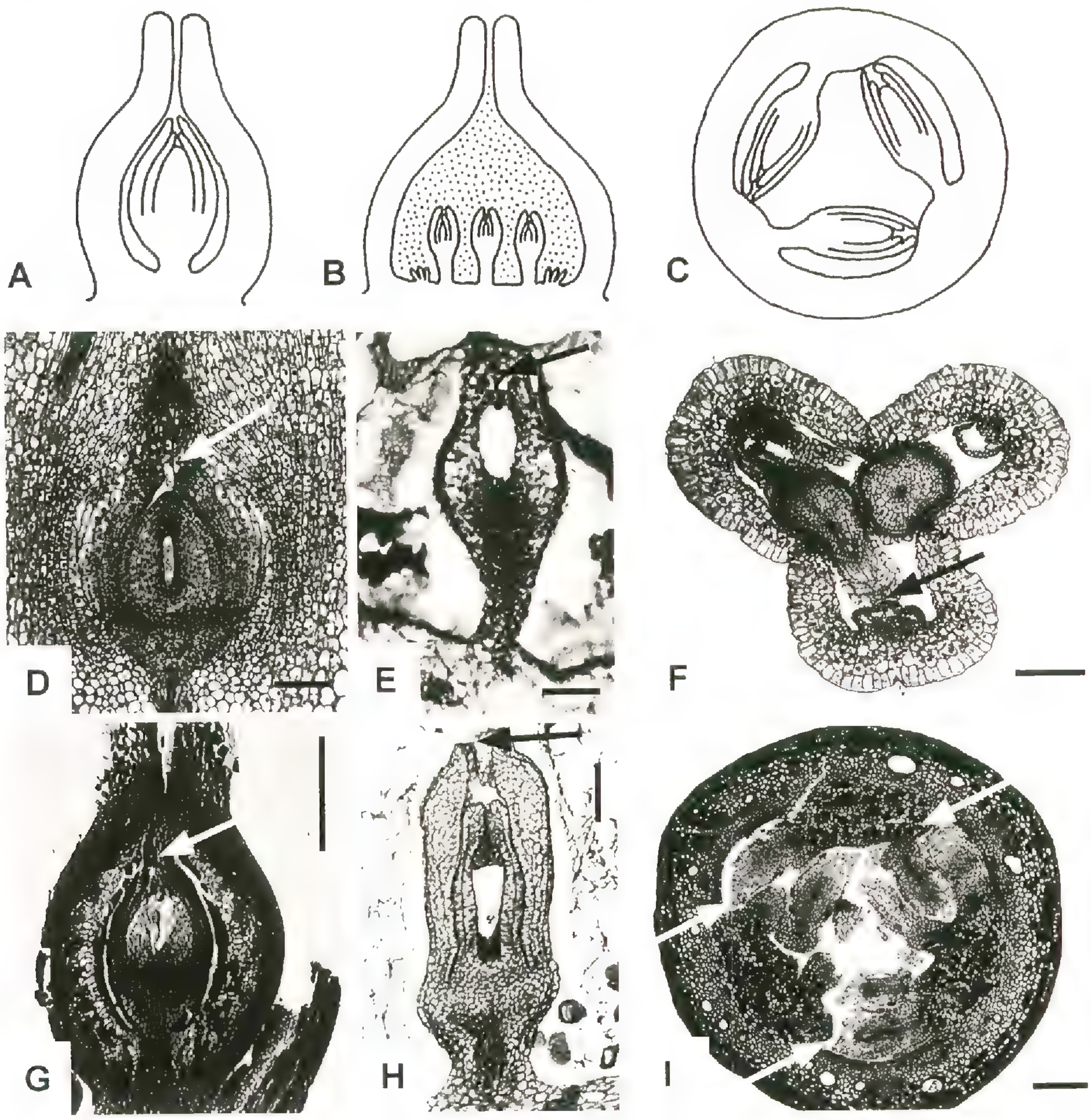


Figure 16. Orthotropous ovules and ovary architecture in angiosperms. —A, D, G. A single ovule at the base of a locule, with micropyle directed to stylar canal. —B, E, H. Several ovules in a spacious locule filled with secretion. —C, F, I. Several ovules in a locule with parietal placentae, with micropyles directed to neighboring placenta. A–C. Schematic figures of ovaries with ovules. D–I. Microtome sections of ovaries with ovules. —D. *Piper augustum* (Piperaceae). —E. *Hydrocharis morsus ranae* L. (Hydrocharitaceae). —F. *Mayaca* sp. indet. (Mayacaceae). —G. *Polygonum filiforme* Thunb. (Polygonaceae). —H. *Pistia stratiotes* L. (Araceae). —I. *Casearia silvana* Schltr. (Salicaceae) (arrows point to micropyle). Magnification bars: D, E, H = 0.1 mm; F, I = 0.2 mm; G = 0.5 mm. (A, B, C from Endress, 1994a. Reprinted with permission from Cambridge University Press.)

where the same organ has exposed and covered areas. For instance, if there is an indument of hairs, the hairs may be restricted to or more strongly developed on the exposed areas (*Stellaria media* (L.) Vill., Nelson, 1954; *Chiranthodendron* Larreat., *Fremontodendron* Coville, von Balthazar et al., 2006; *Rivea* Choisy, Staples, 2007; *Convolvulus tricolor* L., pers. obs.). Another difference is that the exposed flanks are green whereas the covered flanks are hyaline (*Ipomoea purpurea* (L.) Roth., pers. obs.). The most detailed study on this positional effect is that by Warner et al. (2005, in press) for *Nuphar* and other Nymphaeaceae.

Often in imbricate aestivation the margins are thin (two cell layers). The thin part of the covered flanks is often broader than that of the exposed flanks within a flower (e.g., sepals in Caryophyllaceae, Rohweder, 1970; *Corynocarpus* J. R. Forst. & G. Forst., Matthews & Endress, 2004; *Brexia* Noronha ex Thouars; petals in *Siphonodon* Griffith, Matthews & Endress, 2005a; and sepals and petals in *Ixerba* A. Cunn., Matthews & Endress, 2005b). This influence of the position on the differentiation of the sepal flank is especially obvious in pentamerous flowers with quincuncial aestivation. The third-formed sepal of the five has a covered and an exposed flank, and the covered flank is regularly thinner than the exposed flank (Rohweder, 1970). A well-known example are the sepals of rose flowers, in which the exposed margins have pinna-like appendages, whereas the covered margins lack them; Troll

(1957) cites a rhyming Latin riddle on this behavior of rose sepals, which reportedly goes back to Albertus Magnus (13th century).

In valvate tepal, sepal, or petal aestivation, the organs have thick, abruptly ending margins, they are triangular, and the curvature of the margins reflects the shape of the bud—the more curved the margins are, the shorter and rounder the bud. Among floral fossils, the monocot *Mabelia* Gandolfo, Nixon & Crepet has such valvate tepals (Gandolfo et al., 2002).

Sepal Aestivation and Pinnate Petals

Petals that are elaborate and have several lobes along the margins (pinnate shape) tend to occur in flowers with valvate calyx aestivation (Endress & Matthews, 2006). A reason for this correlation may be that in valvate aestivation the margins of two adjacent sepals are contiguous in a mirror symmetrical fashion and do not change position relative to each other during development. Thus, the space for the complicated petal to develop does not change its shape, and the petal lobes are unobstructed in their development. Such pinnate petals are also known from floral fossils (Carpenter & Buchanan, 1993). In these fossils, sepal aestivation is unknown, but it can be predicted that it was probably valvate not only because they appear to belong to Cunoniaceae, which often have valvate sepals (also in cases with simple or no petals), but also because of the presence of pinnate petals. Flowers with such lobed or pinnate petals in combination with a valvate calyx are known from many core eudicot families, especially in rosids (some Myrtaceae, Onagraceae, Rhynchocalycaceae, Anisophylleaceae, Cunoniaceae, Elaeocarpaceae, Celastraceae, Rhizophoraceae; Matthews et al., 2001; Matthews & Endress, 2002, 2004; Schönenberger & Conti, 2003; Endress & Matthews, 2006).

Conclusions

The mechanisms of the imprinting of organ shapes by contiguous neighboring organs are unexplored. From the diversity of cases, it appears that there are different degrees of imprinted shapes. There are directly imprinted shapes (such as in the stamens of *Sphenostemon*), and shapes that just fit in the general architecture and may not be directly shaped during the individual development but were installed during evolution (such as in the floral primordia of *Euptelea*). The stamens of Annonaceae may exhibit different levels of imprinting. A more general level may form the cuneate shape, and a more individual level may form the individual deformations that are different in each single stamen. It may not always be easy to recognize to what extent one or the other is involved.

The observations discussed in this paper should entice further and more detailed studies of interrelationships between the whole and the parts of structural systems in plants. I hope they will also help in the reconstruction of fragmentary fossil material. There are certainly more influences of the whole architecture on the shape of single parts than those shown here, which represent conspicuous examples.

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APPENDIX 1. List of plant specimens, collectors, and collection numbers used for original illustrations. Collection dates are provided in cases without a collection number. All vouchers deposited in Z.

Acsmithia davidsonii (F. Muell.) Hoogland, Cunoniaceae, *I. K. Irvine 1212*

Artabotrys hexapetalus (L. f.) Bhandari, Annonaceae, *P. K. Endress 5288*

Barringtonia calyptrata R. Br. ex Benth., Lecythidaceae, *P. K. Endress 4306*

Cananga odorata Hook. f. & Thomson, Annonaceae, *P. K. Endress 1134*

Carpinus betulus L., Betulaceae, *P. K. Endress 923* (Figs. 11, 15G, H), *3794* (Fig. 13D–F), *3795* (Figs. 12A–H, 13A–C), s.n., 7 IV 1968 (Fig. 15H)

Casearia silvana Schltr., Salicaceae, *P. K. Endress 6019*

Couroupita guianensis Aubl., Lecythidaceae, *P. K. Endress 9393*

Cyathocalyx martabanicus Hook. f. & Thomson, Annonaceae, *P. K. Endress 9372*

Euptelea polyandra Siebold & Zucc., Eupteleaceae, *P. K. Endress s.n.*, summer 1968

Fothergilla major Lodd., Hamamelidaceae, *P. K. Endress 717*

Geissois biagiana F. Muell., Cunoniaceae, *P. K. Endress 9211*

Gillbeea adenopetala F. Muell., Cunoniaceae, *P. K. Endress 4273*

Hamamelis virginiana L., Hamamelidaceae, *P. K. Endress s.n.*, 23 IX 1988

Heisteria parvifolia Sm., Olacaceae, *P. K. Endress 97-11*

Hydrocharis morsus-ranae L., Hydrocharitaceae, *K. Urmi König s.n.*, s.d.

Mayaca sp. indet., Mayacaceae, *P. K. Endress 9504*

Mimosa spegazzinii Pirotta, Leguminosae, *P. K. Endress 9723*

Munroidendron racemosum (C. N. Forbes) Sherff, Araliaceae, *P. K. Endress 96-10*

Piper augustum Rudge, Piperaceae, *P. K. Endress 7890*

Pistia stratiotes L., Araceae, *P. K. Endress 1688*

Platanus orientalis L., Platanaceae, *P. K. Endress 2032*

Polygonum filiforme Thunb., Polygonaceae, *P. K. Endress 5261*

Sphenostemon lobosporus (F. Muell.) L. S. Sm., Sphenostemonaceae, *P. K. Endress 9265*

Thunbergia alata Sims, Acanthaceae, *P. K. Endress 7871*

Thunbergia grandiflora Roxb., Acanthaceae, no voucher (photo of flower in Z)

Trichocladus grandiflorus Oliv., Hamamelidaceae, *W. Rauh & Schlieben 9737*

Wilkiea sp. indet., Monimiaceae, *B. P. M. Hyland 10127*

A FOSSIL RECORD FOR GROWTH REGULATION: THE ROLE OF AUXIN IN WOOD EVOLUTION[1]

Gar W. Rothwell,[2] Heather Sanders,[2] Sarah E. Wyatt,[2] and Simcha Lev-Yadun[3]

Abstract

Appreciation for the role of ontogeny in plant evolution has been heightened by advances in studying development using molecular techniques, with a growing number of specific structural features now understood in terms of the genetic, regulatory, and biochemical mechanisms by which they are produced. Paleontological approaches to plant development provide a vehicle for extending that understanding to the ontogeny and evolution of whole organisms through time. Recent studies have shown that developmentally diagnostic features can be identified in the fossil record, where they represent fingerprints for gene-mediated regulatory pathways. The first paleontological evidence for the regulation of cambial activity via the polar axial flow of auxin consists of circular patterns of tracheary elements above buds and branch junctions in the wood of the 375-million-year-old fossil progymnosperm *Archaeopteris* Dawson. That evidence strongly supports homology of secondary vascular tissues in progymnosperms and seed plants, and monophylesis of the lignophytes (progymnosperms and seed plants). Similar anatomical patterns at the same position have now been identified in the wood of tree-sized fossil equisetophytes and arborescent lycophytes that belong to independent lineages, demonstrating that a similar mechanism involving the regulation of secondary vascular tissue production by the polar axial flow of auxin characterizes those clades as well. These data imply that the wood in lignophytes, lycophytes, and equisetophytes originated in conjunction with the parallel evolution of regulation of secondary tissue production by auxin in each clade. The independent origins of secondary vascular tissue in three major clades of Paleozoic plants sensu Kenrick and Crane (1997) reveal evolutionary patterns that are not represented in the living flora and illuminate promising avenues for combining future paleontological studies with molecular and genetic studies to substantially impact our understanding of the role of developmental regulation in vascular plant evolution.

Key words: Evolution, fossils, polar auxin flow, spiral vascular tissues, wood development, xylem.

The rise of classical genetics during the 1920s and 1930s fostered a fundamental advancement in Darwinian evolutionary theory, with the resulting modern synthesis placing biological evolution on a solid genetic footing (Gould, 1994). As a consequence, the focus of the study of evolution shifted from organisms to populations of organisms, and the definition of evolution was modified from changes in the structure of organisms through time to changes in relative gene frequencies through time (Gould, 1994). This modification of focus is appropriate because it is DNA (not individual organisms) that is modified through time to provide the continuity of biological evolution. Another consequence of the modern synthesis was the separation of studies of evolutionary patterns from studies of the evolutionary process. Molecular phylogenetics notwithstanding, evolutionary patterns are established primarily by changes in organisms through time (a major role of paleontology; Kenrick & Crane, 1997), whereas studies of evolutionary process focus primarily on the population genetics and the population biology of living organisms (Howell, 1998).

Recent advancements in developmental genetics and the rise of molecular developmental biology have, for the first time, allowed us to understand structural features in light of the regulatory mechanisms by which structure is patterned. For example, Reinhardt et al. (2003) have shown how auxin accumulation in the shoot apical meristem stimulates leaf outgrowth and thus controls phyllotaxy. Various works are beginning to reveal gene expression patterns that give rise to morphological variation in leaves (Chuck et al., 1996; Hareven et al., 1996; Chen et al., 1997; Kim et al., 2003; Gleissberg et al., 2005; Harrison et al., 2005).

Because we are now able to ascribe particular structural features of plants to the influence of specific genes, ontogenetic studies from the fossil record have the potential to combine with knowledge of molecular developmental pathways and allow us to infer much more precisely the evolution of tissues, organs, and whole organisms through time. Although at first glance

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paleontology seems to offer us scant potential for inferring plant development at the genetic and biochemical levels, a closer look exposes a sound theoretical and empirical foundation for the approach (Gould, 1977; Rothwell, 1987; Stein, 1993; Sanders et al., 2007) and illuminates an opportunity to reincorporate evolutionary patterns from paleontological studies into the mainstream of evolutionary process. As compared to most animals, the potential for the excellent preservation of sequences of developmental processes in fossil plants is common because the plant skeleton occurs at the cellular level (in the form of the cell wall) instead of at the organismal level (as in most animals). Consequently, virtually all plant cells are readily preserved in the paleontological record (Stewart & Rothwell, 1993; Taylor & Taylor, 1993) and available to studies of developmental biology.

The fossil record of vascular plants provides an impressive array of evidence about the ontogeny of tissues, organs, and whole organisms, as well as for the evolutionary pathways by which organs and species of organisms have evolved (Stewart & Rothwell, 1993; Bateman & DiMichele, 2002; Kenrick, 2002). Traditional ontogenetic studies of fossil plants emphasize topics such as the development of shoot systems (Delevoryas, 1964; Scheckler, 1978), growth architecture of sporophytes (Eggert, 1961; Trivett, 1993; Tomescu et al., 2006), primary and secondary meristems (Good & Taylor, 1972; Cichan, 1985), ovule ontogeny (Rothwell, 1971), and microgametophyte development (Millay & Eggert, 1974; Taylor & Rothwell, 1982). Other paleobotanical studies model apical growth (Stein, 1993), infer leaf blade development in relation to meristematic activity deduced from vein patterns (Boyce & Knoll, 2002), hypothesize regulatory mechanisms for vascular differentiation (Stein, 1993; Boyce & Knoll, 2002), and explore the evolution of meristematic activity in relation to structural changes brought about by experimental studies of gene activity in living plants (Sanders et al., 2007).

These strategies for inferring ontogeny from fossils recently have been extended to the investigation of developmental regulatory mechanisms by which the fossilized structures were produced (Rothwell & Lev-Yadun, 2005). Together with the theoretical approaches introduced above, that study illuminates the exciting potential for developmental regulatory mechanisms to be recognized and characterized from the paleontological record. In the present study, we examine and provide an example of the broad potential for fossil plants to provide developmental regulatory data and to infer the evolution of tissues, organs, and whole organisms. The secondary xylem of Paleozoic fossil lycophytes and equisetophytes has been examined to determine if specific evidence for the regulation of cambial activity by polar axial auxin flow (e.g., circular tracheary elements) extends beyond the lignophytes to other euphyllophytes and to lycophytes (Fig. 1). Circular patterns of tracheary elements are present in lignophytes, other euphyllophytes, and lycophytes but vary considerably in the extent and positions of their development. These data are evaluated in relation to the known positions of deviations of the direction of the polar auxin flow in living vascular plants (Lev-Yadun & Aloni, 1990), with the goal of interpreting the evolution of regulatory mechanisms for secondary vascular tissues across the major fossil clades of vascular land plants.

Materials and Methods

Anatomically preserved fossils of woody lycophytes and equisetophytes preserved in carbonate concretions known as coal balls (Rothwell, 2002) were sectioned in tangential view at nodes using the well-known cellulose acetate peel technique (Joy et al., 1956) to expose the patterns of tracheary elements above branches and wide leaf-trace rays. These include the Middle Pennsylvanian (ca. 310 million years old) lycophyte plant *Paralycopodites brevifolius* (Williamson) DiMichele from the Lewis Creek locality in eastern Kentucky (Phillips, 1980), the Upper Pennsylvanian (ca. 295 million years old) lycophyte plant *Chaloneria cormosa* Pigg & Rothwell from the Steubenville locality in eastern Ohio (Phillips, 1980), and a Middle Pennsylvanian (ca. 310 million years old) species of the equisetophyte morphotaxon *Arthropitys* Goeppert from the Lewis Creek locality. Peels were mounted on microscope slides under cover slips in the mounting medium Eukitt (O. Kinder, Germany; distributed by Calibrated Instruments, Inc., Hawthorne, New York) and examined using transmitted and/or reflected light. Images were captured with Microlumina (Leaf Systems, Inc., Southborough, Massachusetts) and Photophase (Phase One Industries, Copenhagen, Denmark) digital scanning cameras mounted on a copy stand or a Leitz Aristophot (Berlin, Germany) bellows camera, and processed using Adobe Photoshop CS (www.adobe.com). Specimens, peels, and microscope slides are housed in the Ohio University Paleobotanical Herbarium. Microscope slides bear acquisition numbers 18,137–18,148.

Results

Paleontological Evidence for the Developmental Regulation of Wood—Initial Work

Evidence for polar auxin regulation of vascular cambial activity recently has been recognized in the

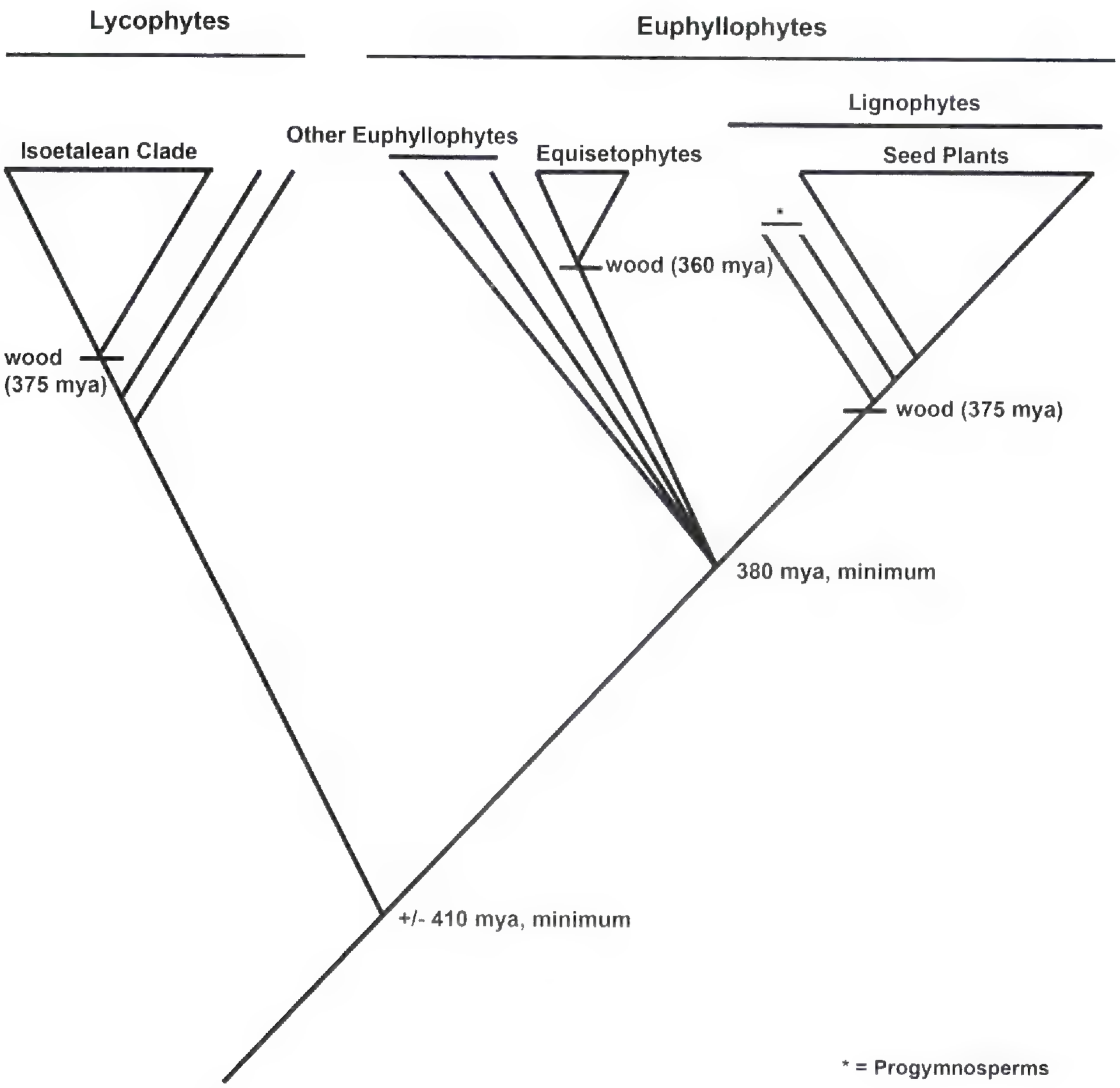


Figure 1. Simplified phylogenetic tree of vascular plants showing currently recognized relationships among lycophytes and euphyllophytes. Euphyllophyte relationships reflect strict consensus of most recent competing hypotheses (Rothwell, 1999; Pryer et al., 2001). Minimum ages for divergence of major clades are placed at relevant nodes. Hash marks on branches indicate oldest evidence for secondary growth in lycophytes, equisetophytes, and lignophytes. Note that the divergence of lycophytes from euphyllophytes occurred at least 35 million years before evidence for the origin of secondary growth in either clade and that lignophytes also appear to have diverged from equisetophytes long before the appearance of secondary vascular tissues in either. (Data primarily from Stewart & Rothwell, 1993; Taylor & Taylor, 1993; Kenrick & Crane, 1997; Rothwell, 1999; and Pryer et al., 2001.)

375-million-year-old tree trunk *Callixylon whiteanum* Arnold (Rothwell & Lev-Yadun, 2005). *Callixylon whiteanum* is a morphospecies for woody stems of the progymnosperm tree *Archaeopteris* Dawson (Trivett, 1993). Tangential sections of *C. whiteanum* wood show circular patterns of tracheids immediately above branches (Fig. 2A) that are comparable to patterns in living seed plants (Rothwell & Lev-Yadun, 2005). Because the circular patterns of living plants are produced by interruption of polar auxin flow above an obstruction in the cambium of woody dicots and conifers (Sachs & Cohen, 1982), such patterns may be regarded as a structural fingerprint for the regulation of vascular tissue development by polar axial auxin flow (Hejnowicz & Kurczyńka, 1987; Lev-Yadun & Aloni, 1990) in *Callixylon* Zallesky and other fossils as well (Fig. 2B).

ISOETALEAN (RHIZOMORPHIC) LYCOPHYTES

Giant, much-branched, tree-sized relatives of the modern quillwart *Isoetes* L. were dominant canopy trees in the equatorial wetlands of the late Paleozoic Era (360–300 million years ago [Ma]; Stewart & Rothwell, 1993). Such plants are commonly assigned to the Lepidodendrales of the isoetalean clade (Stewart & Rothwell, 1993) and are referred to as rhizomorphic because their much-branched rooting system (i.e., rhizomorph) is actually a shoot modified for rooting (Rothwell & Erwin, 1985). In a pioneering

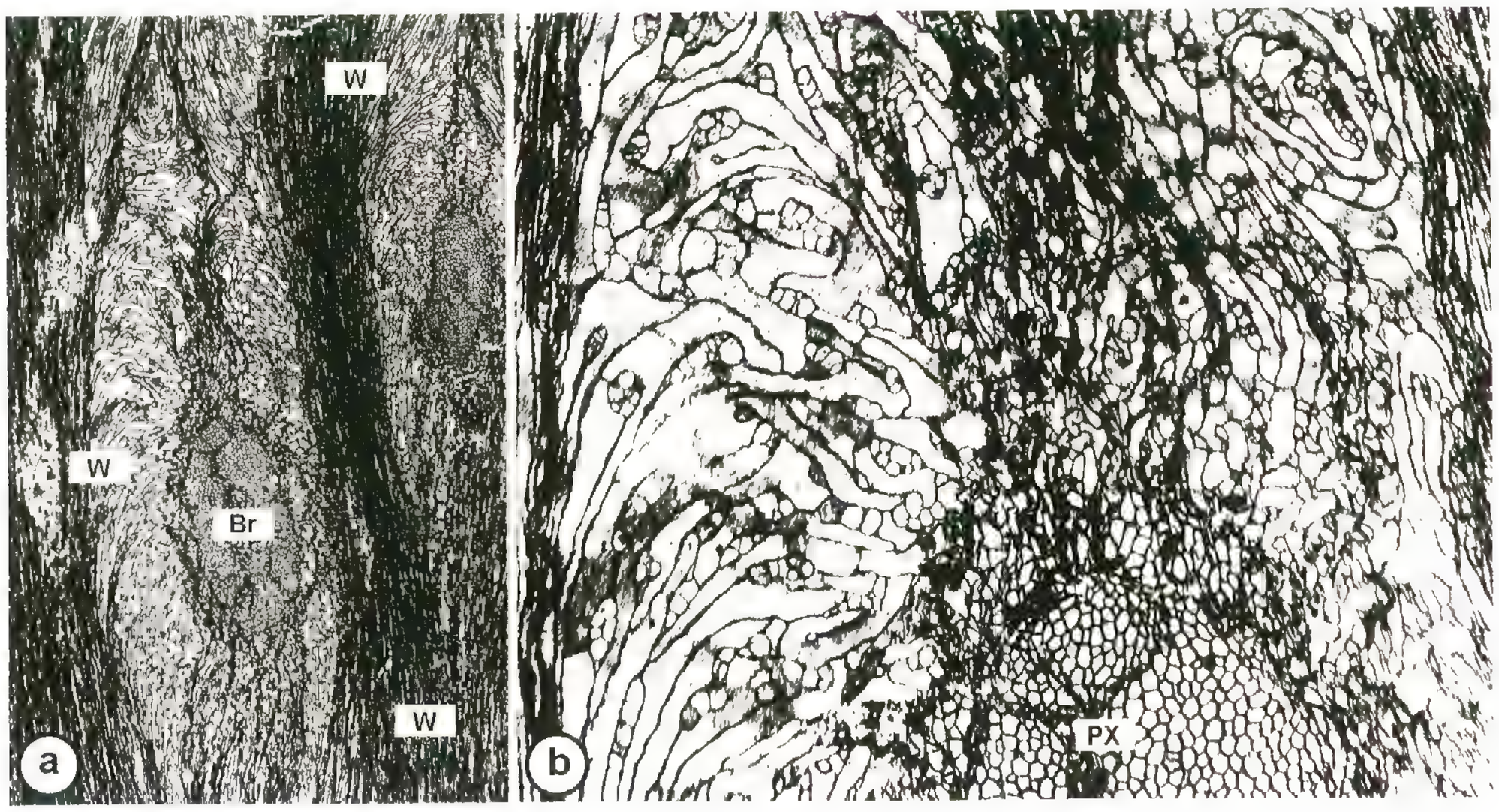


Figure 2. Secondary xylem of a *Callixylon whiteanum* Arnold stem in tangential section at the level of branch divergence. —a. Branch stele (Br) in cross section surrounded by wood (W) of stem, showing circular patterns of tracheids caused by disruption of polar auxin transport and pooling of auxin above branch. —b. Enlargement of wood distal to randomly arranged tracheids of the primary xylem (PX) of the diverging branch stele, showing distorted tracheids and ray cells forming circular patterns.

study of development in fossil plants, Eggert (1961) documented a complex mode of primary and secondary growth for the vascular tissues of lepidodendralean lycophytes (Fig. 3A). Eggert determined that the shoot system of lepidodendralean trees had a primary vascular system that increased in size up to the level of the first branch and then decreased distally (Fig. 3B). By contrast, the thickness of the secondary xylem (i.e., wood; zone with horizontal lines in Fig. 3B) decreases distally (i.e., from the base of the stem), with the apical regions of the branches having no secondary vascular tissues (Fig. 3B). This developmental pattern demonstrates that the wood is the product of secondary growth similar to that of living seed plants in some respects but different in others (Cichan, 1985). Therefore, primary growth and secondary growth are products of independent meristems in arborescent rhizomorphic lycophytes (Eggert, 1961; Cichan, 1985). Up to the present, however, there has been no definitive evidence for the structure or developmental regulation of a lepidodendralean-type vascular cambium.

Paralycopodites brevifolius *(Williamson) DiMichele.* *Paralycopodites* Morey & Morey is a stratigraphically long-ranging genus of arborescent rhizomorphic lepidodendralean lycophytes that is roughly similar to the plant reconstructed in Figure 3A. *Paralycopodites* has a long trunk and much-branched crown, with bisporangiate cones that terminate lateral branches and a highly branched stigmarian rooting system (DiMichele, 1980: but with infrequent dichotomous branching and numerous deciduous lateral branches in the crown as compared to the plant in Fig. 3A). Branches of *Paralycopodites* that have steles in the range of 2–3 cm in diameter (Fig. 4A) display a large pith surrounded by a medulated protostele of primary tracheids with a smooth outer margin, and abundant secondary xylem that comprises a relatively dense wood composed of tracheids and infrequent narrow rays (Fig. 4B). At the level where a branch base diverges from the stem stele, the xylem of the branch consists of a C-shaped stele of tracheids and adaxially oriented parenchyma (Fig. 4B) that becomes a radial stele at more distal levels (DiMichele, 1980). Tangential sections of *Paralycopodites* stems near the periphery of the wood (i.e., along line c–c of Fig. 4A) show regularly arranged, long, straight tracheids and interspersed narrow rays (Fig. 4B, at left and bottom). Immediately distal to the branch trace the axial files of tracheids are disrupted, many tracheids are distorted, and others extend obliquely or horizontally (Fig. 4B, at upper right). By contrast, tracheids immediately below the branch form regular axial files that are similar in pattern to those in non-branching regions (Fig. 4B, at bottom right).

Chaloneria cormosa *Pigg & Rothwell.* Compared to *Paralycopodites* and other lepidodendralean trees, *Chaloneria cormosa* is a relatively small and unbranched rhizomorphic lycophyte of Upper Pennsylvanian age that grew to about 2 m tall (Fig. 3C).

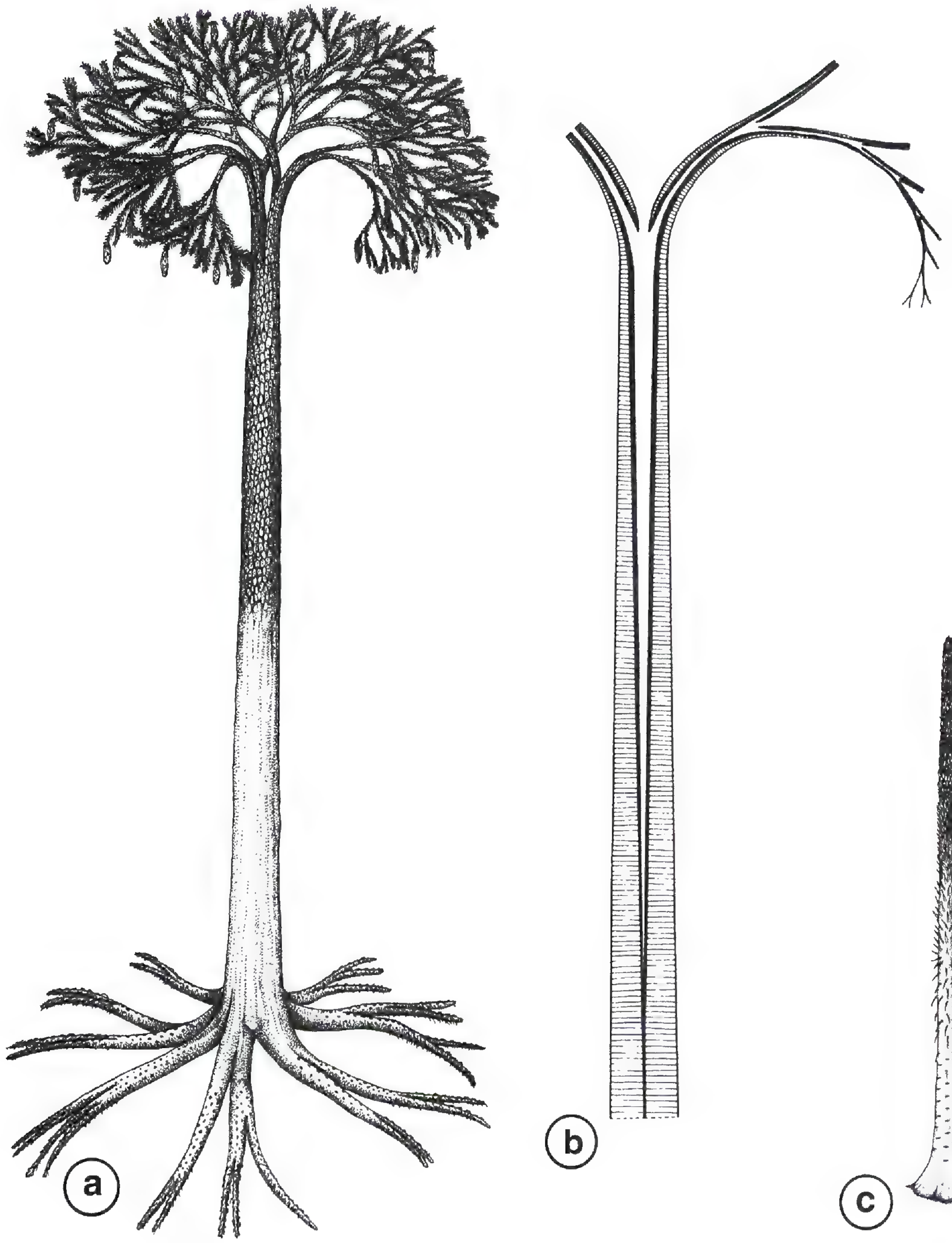
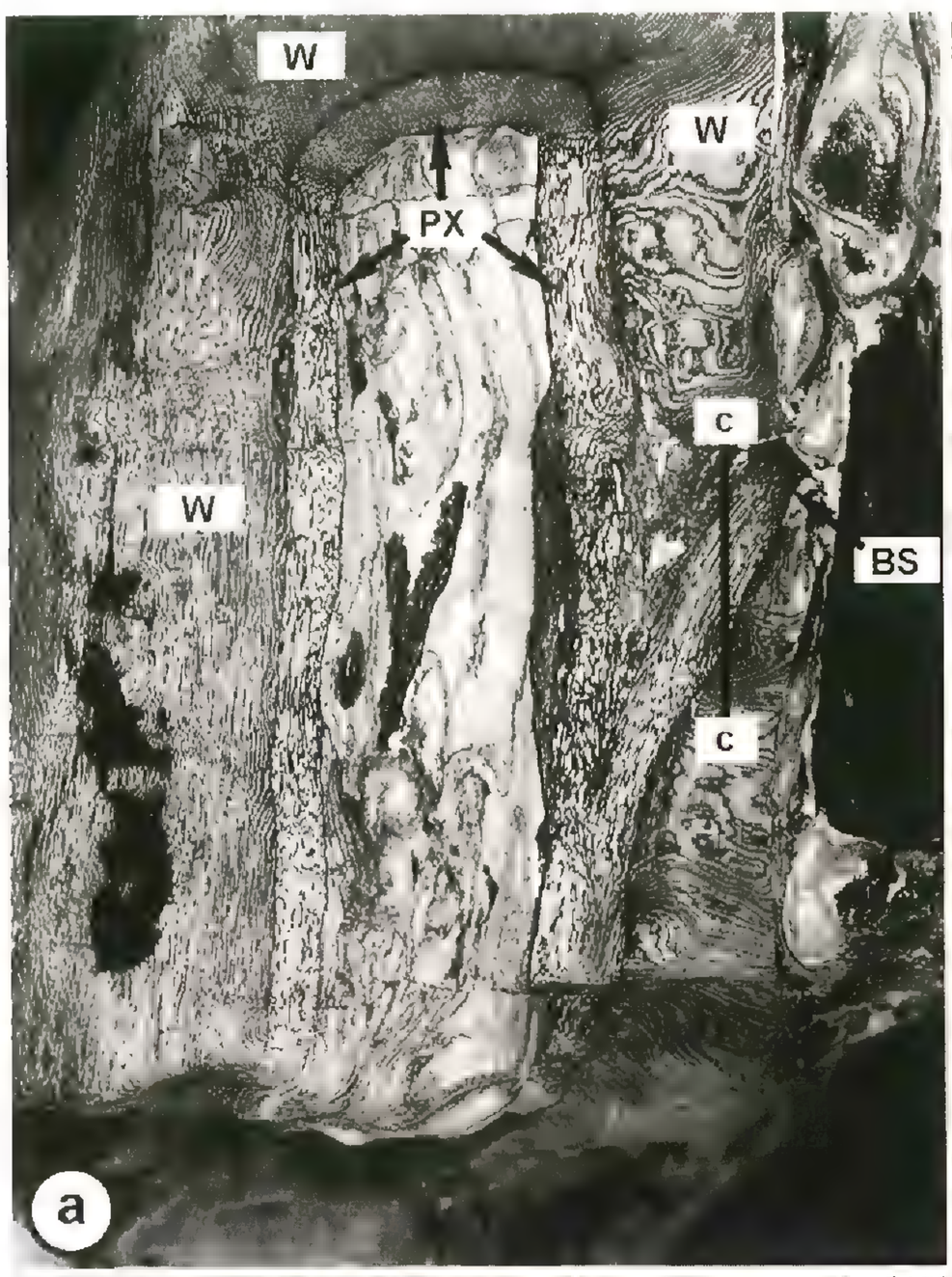
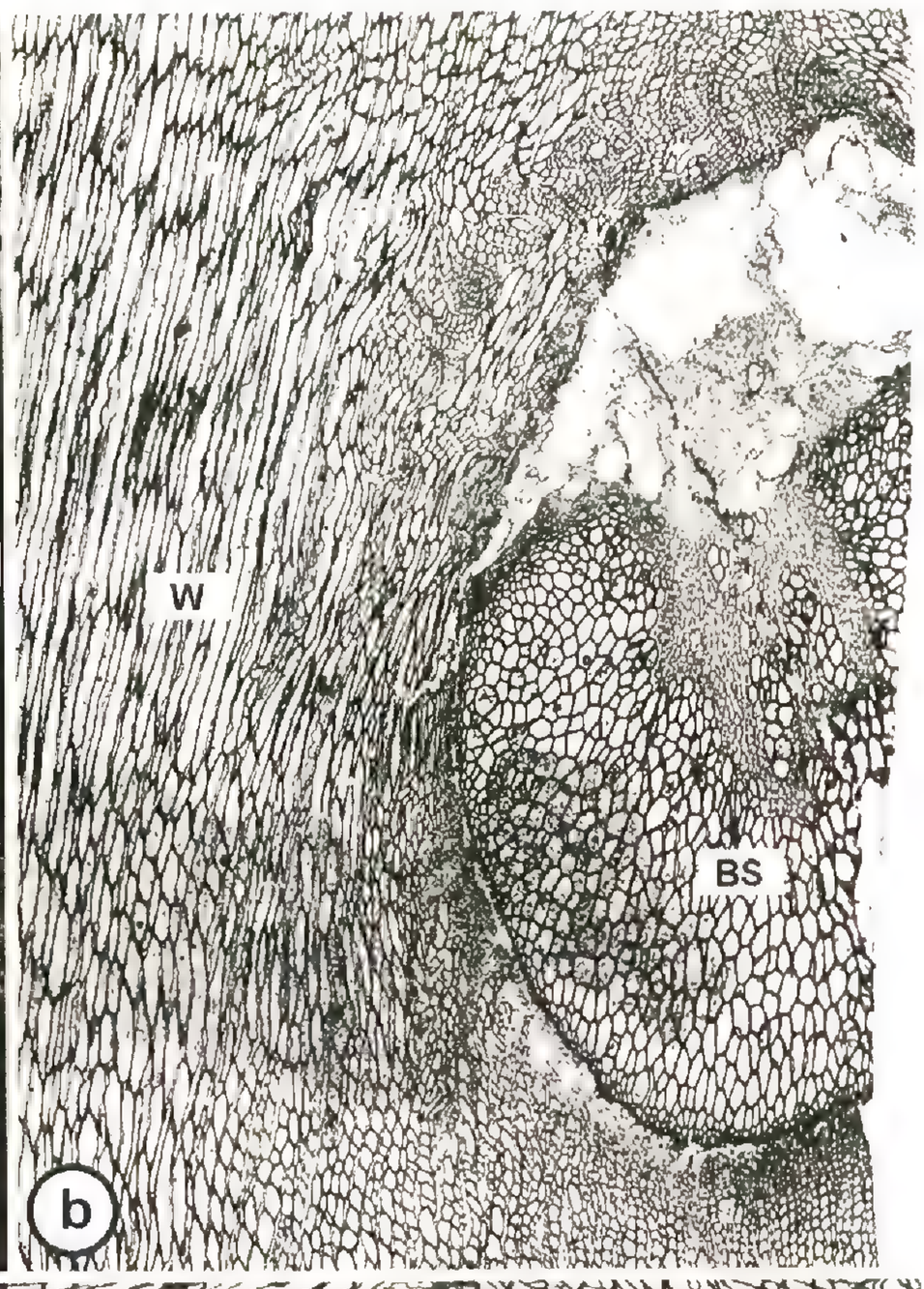
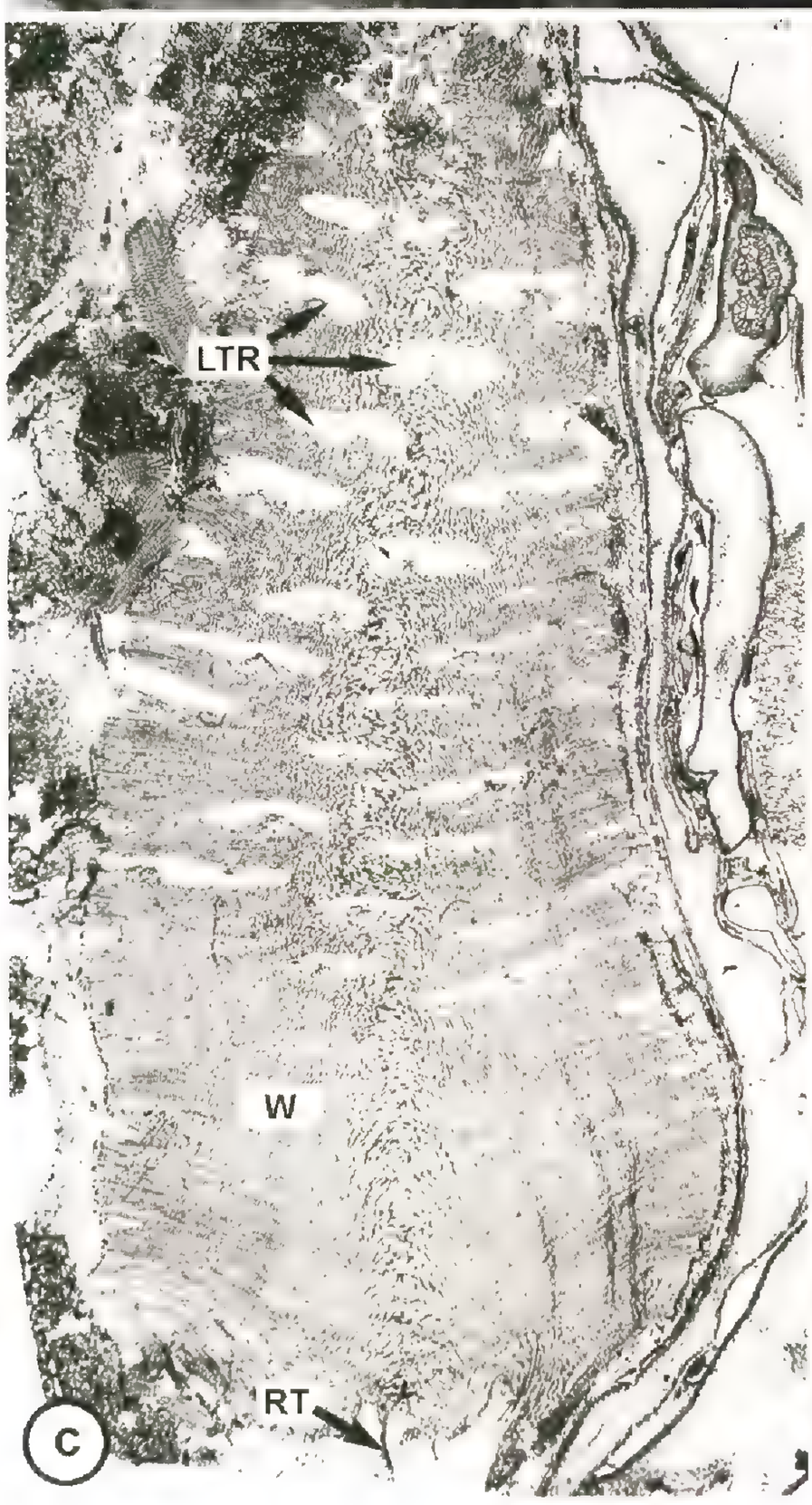


Figure 3. Diagrams of fossilized woody lepidodendralean and isoetalean lycophytes from the Pennsylvanian of North America and Europe. —a. Lepidodendralean tree ca. 8–10 m tall, showing elongated trunk and branching crown with terminal cones and branched rhizomorphic rooting system assignable to the morphogenus *Stigmaria* Goeppert. Modified from Stewart & Rothwell, 1993. —b. Diagrammatic representation of growth architecture of lepidodendralean trees. Note that the size of the primary xylem (black = primary xylem; white = pith) increases up to first branch and then decreases distally with each subsequent branch. Also note that the thickness of secondary xylem (horizontal lines) decreases from base of stem distally. Modified from Eggert, 1961. Used with permission from Cambridge University Press. —c. Unbranched plant of the isoetalean *Chaloneria cormosa* Pigg & Rothwell ca. 2 m tall, showing cormose base, vegetative basal region of stem, and fertile apex. Modified from Pigg and Rothwell, 1981.

This species produced vegetative leaves toward the base, displayed alternating zones of microsporangiate and megasporangiate sporophylls distally, and had a corm-like base similar to the living quillwort, *Isoetes* (Fig. 3C; Pigg & Rothwell, 1981). Secondary xylem occurs at and near the base of the *Chaloneria* Pigg & Rothwell plant (Fig. 4C), but the secondary xylem diminishes in thickness rapidly and is absent from all but the most basal regions of the stem (Pigg & Rothwell, 1981).







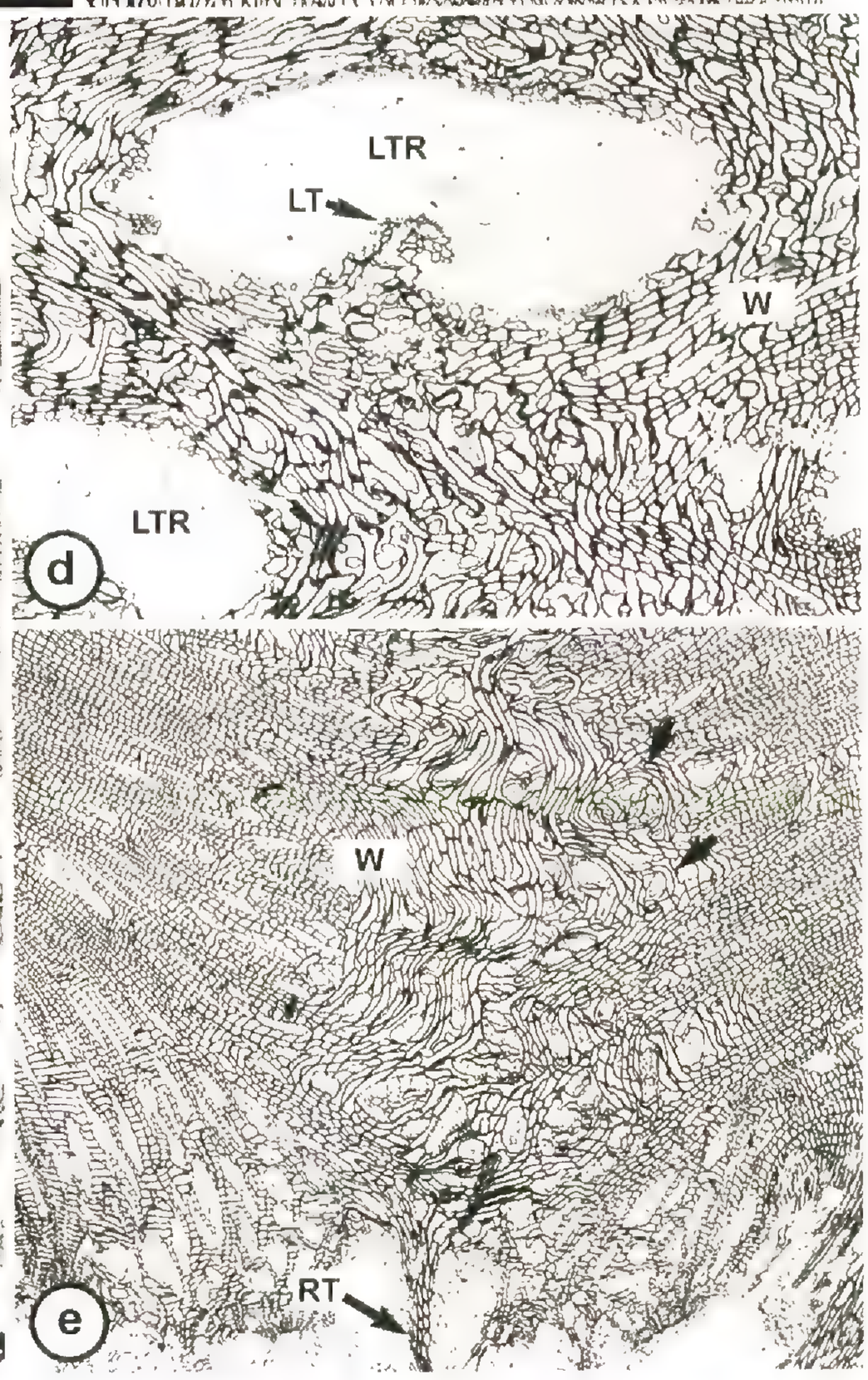


Figure 4. Anatomical sections through stems of fossilized woody rhizomorphic lycophytes showing evidence for axial polar auxin flow in regulation of secondary vascular tissue production. —a. *Paralycopodites brevifolius* (Williamson) DiMichele stem from the lower Middle Pennsylvanian of eastern Kentucky, showing radial view through level of a branching at coal ball surface. The pith region is surrounded by a cylinder of primary xylem (PX; also seen in cross section at the top and bottom), which is enclosed in a cylinder of wood (W). Note diverging branch stele (BS) at right. Line c–c indicates plane of section shown in part c. —b. Tangential section through secondary xylem of stem, cut through line c–c of part a. Note that the

Tangential sections near the base of a *Chaloneria* stem show secondary xylem that is perforated by helically arranged leaf traces (Fig. 4C), each within a broad ray (Fig. 4D). At the base of the plant (Fig. 4C), root traces diverge from the rounded bottom of the woody zone (Fig. 4E, at "RT"). An absence of leaf traces at the base of the plant indicates that leaves were not produced in that region (Fig. 4C, at bottom; 4E). Tangential sections through the secondary xylem of *Chaloneria* reveal radial files of tracheids that follow a sinuous course around the large leaf-trace rays (Fig. 4C, D). In some areas, particularly immediately distal to each ray, the tracheids are highly distorted, with some tracheids being branched and others forming circular patterns (Fig. 4D). Highly distorted patterns of tracheids are also present at the base of the plant, where branched tracheids and swirls of tracheids are common (Fig. 4E, at arrows).

ARBORESCENT WOODY EQUISETOPHYTES

Arborescent equisetophytes of the Pennsylvanian coal swamps were essentially giant woody *Equisetum* L.-like plants (Fig. 5A; Good, 1975) that produced wood that is assignable to three morphogenera including *Arthropitys* (Taylor & Taylor, 1993). In cross sections, *Arthropitys* stems have a large pith surrounded by a ring of primary xylem bundles (Fig. 5B). Conspicuous carinal canals characterize the primary xylem and vallecular canals occur in the cortex (Figs. 5B, 6A), both of which are virtually identical to comparable structures in living species of *Equisetum* (Fig. 6D). By comparison to *Equisetum*, *Arthropitys* stems range much larger in diameter. *Arthropitys* stems also differ from *Equisetum* by the production of abundant secondary xylem that forms wedges of radially aligned tracheids separated by wide parenchymatous rays (Figs. 5B, 6A; Stewart & Rothwell, 1993).

At the level of a node, tangential sections through the secondary xylem of *Arthropitys* stems show branch bases that are positioned slightly above and on alternating radii with leaf traces (Fig. 6B). The branch base shows a large hollow pith that is surrounded by a ring of carinal canals and wood, all of which are similar to those in the stem from which the branch has arisen (cf. Fig. 6A, B). As seen in tangential sections, the secondary xylem of the stem typically consists of regular axial files of long and straight tracheids and interspersed narrow rays (Fig. 6B, at sides) except where leaf traces and especially branch junctions are positioned. Tracheids of the secondary xylem extend around leaf traces (Fig. 6B, at bottom arrows), often showing a small area of tracheids that form a circular pattern (Fig. 6C, at bottom left).

Evidence of regulation of vascular differentiation by the axial polar auxin flow is more pronounced where the wood extends around the much larger branch bases (Fig. 6B). Cells distal to and extending around to the sides of the branch stele form distinct swirls and circular patterns (Fig. 6C), and there are much larger numbers of ray cells than in other areas of the secondary xylem. Also, some tracheids branch (Fig. 6B, arrow at top).

SYSTEMATIC SCOPE OF POLAR AUXIN REGULATION IN WOOD DEVELOPMENT

Fossil wood assigned to the genus *Callixylon* (Fig. 2A, B) is produced by the plant *Archaeopteris* (Beck, 1960), which is a member of the Progymnospermopsida, a paraphyletic grade subtending seed plants in the clade that is known as lignophytes (Fig. 1; Crane, 1985; Rothwell & Serbet, 1994). The discovery of a common regulatory pathway for the development of secondary vascular tissues in progymnosperms and seed plants strengthens the hypothesis that such tissues are homologous and provides additional support for the definition of the lignophyte clade (Fig. 1). Although large woody trees with substantial secondary vascular tissue production are restricted to seed plants in the modern flora, the fossil record includes highly branched, woody equisetophyte and lycophyte trees that were prominent components of wetland forests during the Paleozoic Era (Taylor & Taylor, 1993).

Data developed in the current study reveal cellular patterns in the secondary xylem of both Paleozoic lycophytes (*Paralycopodites* and *Chaloneria*) and equisetophytes (*Arthropitys*) that are diagnostic for regulation of cambial growth and secondary xylem production by polar axial auxin flow among living

←

tracheids distal to the branch stele (BS) are distorted, whereas those below the branch stele are not. —c. Tangential section at base of *Chaloneria cormosa* Pigg & Rothwell from the Upper Pennsylvanian of Ohio, showing helically arranged leaf traces with large leaf-trace rays (LTR) distal to a cormose plant base. Note that no leaf traces or rays traverse the wood (W) at the base of the plant. —d. Enlargement of wood in stem region of part c (W), showing diverging leaf trace (LT) surrounded by a wide LTR. Note contorted and circular patterns of tracheids distal to the LTR. —e. Enlargement of part c at base of plant showing distorted and circular patterns of tracheids (at arrows) in the wood (W) above a diverging rootlet trace (RT) at the base of the plant.

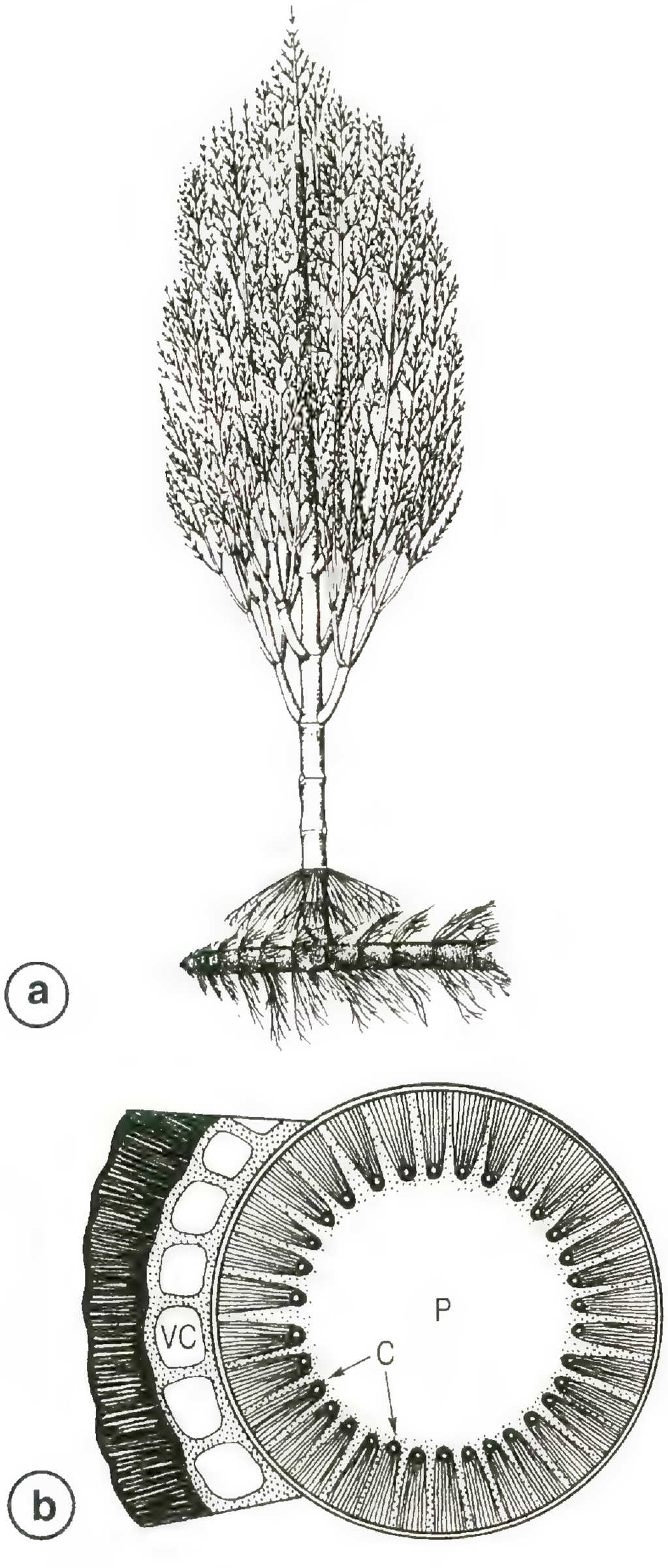


Figure 5. Diagrams of fossilized calamitalean equisetophytes from the Pennsylvanian of North America and Europe. —a. Calamitalean tree ca. 6–8 m tall, diverging from underground rhizome. Modified from Stewart and Rothwell, 1993. —b. Cross section of *Arthropitys* stem showing characteristic *Equisetum*-like anatomy and secondary xylem. Important features include hollow pith (P), carinal canals (C) surrounded by wood, and vallecular canals (VC) in cortex. Modified from Stewart and Rothwell, 1993.

seed plants. Distorted files of axial secondary xylem cells, branched tracheids, and circular patterns of tracheids are associated with leaf trace divergence in *Chaloneria* (Fig. 4C, D), reflecting swirling polar auxin currents above disruptions in the vascular cambium that are every bit as pronounced as those that result from obstructions to auxin flow in the cambium of living and fossil lignophytes (Fig. 2). These patterns document that axial polar auxin flow was involved in the regulation of wood development in lycophytes as well as in lignophytes (Fig. 1; Rothwell & Lev-Yadun, 2005). Likewise, the occurrence of well-developed diagnostic cellular patterns in the secondary xylem of *Arthropitys* demonstrates the regulation of secondary xylem development by the axial polar auxin flow in extinct equisetophytes. Together, these data from lignophyte, equisetophyte, and lycophyte taxa reveal that polar auxin transport facilitates a common mode of developmental regulation for secondary vascular tissues across the vascular plant evolutionary tree (Fig. 1). That is to say, polar auxin regulation of wood development is common to both major clades of living vascular plants, Lycophytina and Euphyllophytina (which includes both equisetophytes and lignophytes; Fig. 1).

Variations in Fossil Evidence for Regulation by Axial Polar Auxin Flow

Although anatomical evidence for the regulation of secondary vascular tissue production by axial polar auxin flow is present in all three major clades of vascular plants that have produced abundant wood through time (i.e., lignophytes, equisetophytes, lycophytes; Fig. 1), there are significant variations in the degree of development and in the locations of evidence among representatives of the various clades. In all of the examples studied to date, evidence for regulation of vascular differentiation by deviation from the typical orientation of the axial polar auxin flow is preserved in the secondary xylem above the positions where there would have been obstructions to auxin flow in the cambium during tissue patterning (Figs. 2, 4, 6). Lignophytes (Fig. 2B), such as the extinct progymnosperm *Archaeopteris* (represented here by the morphotaxon *Callixylon*, Fig. 2), living species of both conifers and dicotyledonous flowering plants (Rothwell & Lev-Yadun, 2005: figs. 2, 3), and fossil equisetophytes such as *Arthropitys* (Fig. 6B) all show obvious regions of distorted tracheids, branched tracheids, and circular patterns of cells distal to branch junctions. The equisetophytes *Arthropitys* and *Archaeocalamites* Stur also show evidence of circular patterns associated with leaf trace divergence (Fig. 6B; Smoot et al., 1982), demonstrating that small obstructions in polar auxin regulation were sufficient to produce the diagnostic fingerprint in this clade by the Mississippian Period (ca. 330 Ma).

Files of axial tracheids of the secondary xylem distal to branches in *Paralycopodites* are also disrupted above the branches, but the extent of the zone is much smaller and the distortions are far less pronounced (Fig. 4B, at

top) than in the other plant clades studied. This subtle evidence for regulation of secondary xylem differentiation by the axial polar auxin flow in *Paralycopodites* is most easily recognized by noting differences in the wood immediately above and below a branch. By comparison to the subtly distorted patterns distal to a branch, files of cells below the same branch (cf. cells above and below the branch in Fig. 4B) of *Paralycopodites* show no evidence of distortion at all. Were it not for the obvious disruptions of cell files with branched tracheids and circular patterns of cells present in the wood of other lycophytes (e.g., *Chaloneria*; Fig. 4D, E), it would be difficult to interpret regulation of secondary xylem development by the axial polar auxin flow in fossil isoetalean lycophytes with certainty.

Chaloneria cormosa is unbranched and smaller than lepidodendraleans (cf. Fig. 3). *Chaloneria* also has a cormose base like living *Isoetes*, rather than the elongated and much-branched stigmarian rooting system that characterizes *Paralycopodites* and other members of the Lepidodendrales (Fig. 3A). Nevertheless, evidence for the regulation of secondary vascular tissue development by the axial polar auxin flow is well represented in *Chaloneria* (Fig. 4C–E). Disrupted cellular patterns, branched tracheids, and swirling patterns of tracheids occur in association with the wide rays that accompany diverging leaf traces (Fig. 4C, D). Because of the extremely short internodes and numerous helically arranged leaves of *Chaloneria* (Fig. 4C), the closely spaced wide leaf-trace rays must have produced a considerable obstruction to the axial polar auxin flow in the developing vascular cambium. That complex pattern of obstruction probably accounts for the occurrence of contorted and branched tracheids around several sides of many rays (Fig. 4D).

One of the most distinctive and initially unexpected occurrences of an axial polar auxin flow fingerprint in fossils is found at the base of the *Chaloneria* plant, where particularly well-developed circular patterns are present (Fig. 4C, E). Such patterns were first recognized as being distinctive and bilateral in distribution before the role of the axial polar auxin flow in regulation of secondary xylem development was understood (Pigg & Rothwell, 1979). Because those circular patterns are present in a region that is below all typical obstructions in polar auxin flow that would have been caused by the divergence of branch junctions and leaf traces, their occurrence must be attributable to another phenomenon. We know from wounding and partial girdling experiments in oak (*Quercus ithaburensis* Decne. and *Q. calliprinos* Webb) that circular vascular tissues are also formed above any large obstacle to the polar axial auxin flow in tree trunks (Lev-Yadun & Aloni, 1991). An understanding of the unique morphology of rhizomorphic lycophytes provides a plausible explanation for the formation of the circular tissues at the base of *Chaloneria* plants.

Although some questions about the homologies of the rooting system of *Isoetes* remain among authors who work primarily with living species (Yi & Kato, 2001), there now is overwhelming evidence that the bipolar growth and rooting systems of isoetalean lycophytes are unique among all vascular plants. Evidence from the developmental morphology of *Isoetes* (Karrfalt & Eggert, 1984) and from the structure, development, and embryology of fossil species (Frankenburg & Eggert, 1969; Phillips, 1979; Karrfalt, 1980) supports the hypothesis that isoetalean lycophytes have no true roots that could be homologous to the roots of *Selaginella* P. Beauv., other non-rhizomorphic lycophytes, or any other group of vascular plants (Rothwell & Erwin, 1985). Rather, the rhizomorph of *Isoetes* and all of the isoetalean lycophytes is homologous to a shoot system that has become highly modified for rooting the plant. This means that the structures commonly referred to as the roots of *Isoetes* are actually leaves that have been modified for rooting (Frankenburg & Eggert, 1969).

That interpretation is both explained by and further strengthened by the distinct evidence for disruption in polar auxin flow at the very base of the *Chaloneria* plant. If, indeed, *Chaloneria* and other rhizomorphic lycophytes have no true roots, as interpreted from the fossil record (Frankenberg & Eggert, 1969; Rothwell & Erwin, 1985), then basally flowing auxin would have nowhere to go after reaching the cormose base of the plant. The auxin must have pooled and swirled in the cambial zone at the base of the trunk, thus inducing the distinctive anatomical patterns in the wood at the base of *Chaloneria* specimens (Fig. 4C, E; Pigg & Rothwell, 1979). Anatomical descriptions of the basal regions of other fossil rhizomorphic lycophytes with cormose bases are scarce. However, in a genus that is even more ancient than *Chaloneria* (i.e., the 360-million-year-old Lower Carboniferous lycopohyte *Trabicaulis flabellilignis* Meyer-Berthaud; Meyer-Berthaud, 1984), there is evidence for disruptions of tracheid patterning similar to those of *Chaloneria* both above leaf trace divergences (i.e., Meyer-Berthaud, 1984: fig. 8) and at the base of the plant (i.e., Meyer-Berthaud, 1984: Planch 3, fig. 4). These data reveal that regulation of secondary vascular tissue production by the axial polar auxin flow was probably widespread among rhizomorphic lycophytes with cormose bases. Moreover, there is now additional compelling evidence that the most distinctive synapomorphy for isoetalean lycophytes, the rhizomorph, represents a shoot system that has been modified for rooting.

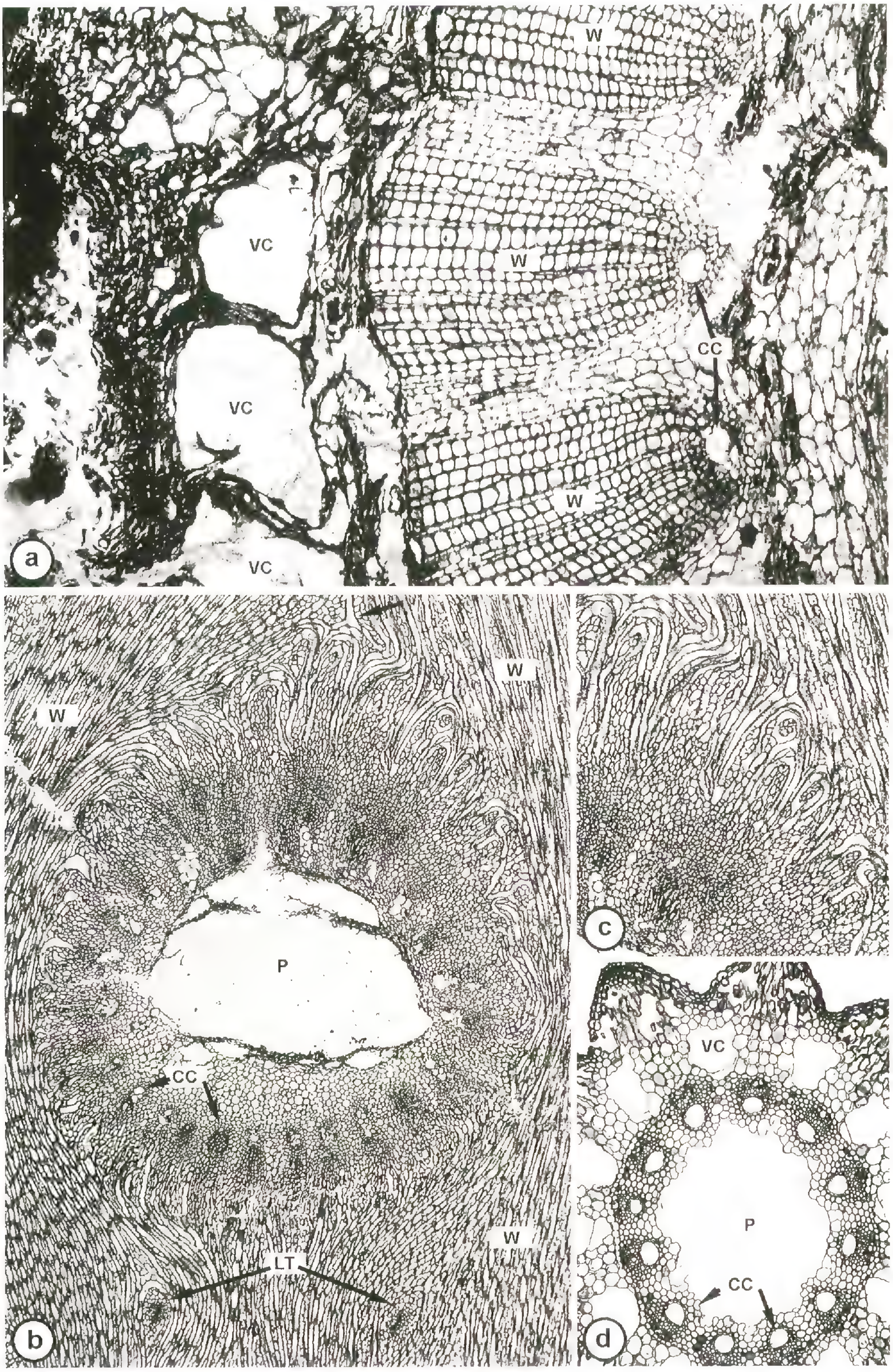


Figure 6. Anatomical sections through stems of large, woody equisetophytes assignable to the Paleozoic Calamitales (a–c) and living *Equisetum* L. (d). —a. Cross section through segment of *Arthropitys* Goeppert stem from Westphalian A sediments of England, with pith at right and cortex at left. Carinal canals (CC) in the primary xylem and vallecular canals (VC) in the cortex that are comparable to those of living *Equisetum* (d). Note the well-developed segments of secondary xylem that are separated by wide pith rays. This wood (W) shows both long radial files of secondary tracheids and interspersed narrow parenchymatous rays. —b. Tangential section through wood of *Arthropitys* at the level of a node. Diverging branch base in cross section shows a

Fossil Evidence for Growth Regulation in the Evolution of Plants

Methodology for recognizing specific developmental regulatory pathways from diagnostic structural features of fossil plants has recently begun to emerge as an additional approach for improving the understanding of evolution through geological time and for testing hypotheses of evolution and phylogenetic relationships among major clades of vascular plants (Rothwell & Lev-Yadun, 2005). This represents a new approach, the first application of which is based on our knowledge that there are disrupted files of contorted and branched cells that form circular patterns immediately distal to branches and large leaf-trace rays in living seed plants. Because those patterns are known to represent an anatomical fingerprint for the axial polar auxin flow that regulates patterns of secondary vascular tissue development (Sachs & Cohen, 1982; Hejnowicz & Kurczyńska, 1987; Lev-Yadun & Aloni, 1990), the discovery of circular patterns in the wood of a 375-million-year-old species of *Archaeopteris* has provided the first direct evidence that progymnosperms and seed plants share a common mechanism of growth regulation for secondary vascular tissues. Those data also reveal that auxin-mediated secondary vascular tissue development is a synapomorphy for lignophytes (i.e., a clade that includes seed plants and their progymnospermous sister groups).

Implications for the Evolution of Wood Across Vascular Plants

These newly developed data for the fossil evidence for regulatory mechanisms of wood production now can be combined with an understanding of phylogenetic pattern and first occurrences of secondary vascular tissues in divergent clades to develop hypotheses for the evolution of secondary vascular tissues across the vascular plant evolutionary tree. Although the overall topology of vascular plant relationships remains a subject of controversy (Rothwell & Nixon, 2006), there is wide agreement about some of the deep internal nodes of the evolutionary tree. Living plants and their closest extinct sister groups comprise two major clades, Lycophytina and Euphyllophytina sensu Kenrick and Crane (1997), some representatives of which can be identified as early as the Late Silurian. This places the minimum age for divergence of lycophyte ancestors from euphyllophyte ancestors at about 410 million years, which is at least 35 million years earlier than the first occurrence of secondary vascular tissues in either clade (Fig. 1). Therefore, secondary vascular tissues in lycophytes and euphyllophytes must have evolved independently. Evidence for separate origins of secondary vascular tissues in equisetophytes and lignophytes is not as strong because of the current uncertainty about relationships among euphyllophytes (Fig. 1; Rothwell & Nixon, 2006). However, the first evidence for secondary vascular tissues in either equisetophytes or lignophytes is on the order of five million years younger than the minimum age of divergence between these two clades (Fig. 1). Therefore, it is also likely that secondary vascular tissues evolved independently in the ancestors of *Equisetum* and seed plants.

We now recognize that the developmental mechanism (i.e., involving polar axial auxin flow) for secondary vascular tissue production is common to lignophytes, equisetophytes, and lycophytes, but that the secondary vascular tissues evolved separately either in lycophytes and euphyllophytes or in all three clades (Fig. 1). Therefore, wood production in the major clades of vascular plants is the product of convergent evolution. The question remains whether the underlying regulatory mechanism (i.e., by the axial polar auxin flow) for secondary vascular tissues also evolved independently in the various clades or whether it was present in the genomes of all of the clades prior to the evolution of secondary vascular tissues.

Among living plants, this question has been addressed in a molecular survey of embryophytes and their algal sister groups (Cooke et al., 2002). Although conjugation rates and the complexity of auxin metabolism both increase toward the distal branches of the tree, these authors record that indoleacetic acid (IAA) metabolism is present in the bryophytic sister groups of vascular plants as well as all of the lycophytes and euphyllophytes that have been surveyed. Within the tracheophytes, auxin

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←

hollow pith (P) surrounded by pith parenchyma and a ring of carinal canals (arrows at CC). Branch is situated just distal to, and on a radius that alternates with, radii upon which leaf traces (arrows at LT) are produced. Note that circular patterns of tracheids (indicative of the role of the axial polar auxin flow in regulation of secondary vascular tissue production) are positioned distal to the branch, but are not produced below the branch. Arrow at top of photo identifies branching tracheid. —c. Enlargement of disrupted wood pattern distal to branch in part b, showing swirls and circular patterns of cells. —d. *Equisetum arvense* L. stem in cross section, showing anatomical features (CC = carinal canal, P = pith, VC = vallecular canal) that are virtually identical to the primary tissues of *Arthropitys* (parts a and b).

metabolism is widely associated with primary vascular tissue production (Leyser & Berleth, 1999); it also may be associated with development of conducting tissues in bryophytes (Cooke et al., 2002).

The basal/apical ratio of auxin flow is one to two orders of magnitude lower in the only lycophyte studied, *Selaginella*, than it is in the euphyllophytes studied (Cooke et al., 2002: table 4). If that difference is characteristic of lycophytes as a whole, it would account for the much less obvious anatomical fingerprint for the role of axial polar auxin flow in the regulation of secondary xylem formation above the branches of *Paralycopodites* than in similar positions of the fossil euphyllophytes studied (i.e., *Archaeopteris* and *Arthropitys*). This ratio has yet to be determined for *Equisetum*. However, if the distinctive fingerprint present in the wood of *Arthropitys* (Fig. 6B) is correlated with levels of polar auxin flow, we hypothesize that future studies will document polar auxin metabolism in equisetophytes that is comparable to other euphyllophytes.

Conclusions

Until the recent recognition of structural fingerprints for axial polar auxin flow in fossil plants (Rothwell & Lev-Yadun, 2005), it has not been possible to employ data from the paleontological record to infer plant evolution from changes in developmental regulation. However, the discovery of developmentally diagnostic circular patterns in fossil secondary xylem reveals that secondary vascular tissue development was probably regulated by the axial polar auxin flow in all of the major clades of vascular plants that have produced woody trees through time. Because such patterns result from obstructions of the axial polar auxin flow in the vascular cambium, both the positions of the circular patterns and the extent of their development provide meaningful information about specifics of the auxin metabolism, the growth and development that has resulted from regulation by auxin, and the organography of the extinct plants. These data imply that the much lower basal/apical IAA transport ratios found in living *Selaginella* also characterized Paleozoic fossil lepidodendraleans and that the patterns preserved above branch junctions of Paleozoic fossil Calamitales are predictors of the regulation by the axial polar auxin flow in living *Equisetum* that is similar to other modern euphyllophytes. Likewise, the extensive development of circular patterns at the base of *Chaloneria* and similar Paleozoic lycophytes with a plant base similar to living *Isoetes* strongly supports the hypothesis that rhizomorphic lycophytes are rooted by a highly modified shoot system and that they lack true roots that are homologous to those of other vascular plants.

Although the fossil record now provides strong anatomical evidence that a common mode of developmental regulation underlies secondary vascular tissue production in all of the major clades of vascular plants that have produced extensive wood through time, the currently recognized pattern of phylogeny for tracheophytes reveals that secondary vascular tissues probably evolved independently in lycophytes, equisetophytes, and lignophytes. The occurrence of regulation by polar auxin flow of primary growth across the entire spectrum of vascular plants (Sachs, 1991; Scarpella et al., 2006) indicates that genes for this developmental regulatory mechanism were present in the herbaceous ancestors of all woody plants, although they were not expressed in circular patterns before the emergence of secondary vascular tissues (Rothwell & Lev-Yadun, 2005) and were available for exaptation to the new role of secondary vascular tissue development. Therefore, while the secondary xylem of lignophytes, equisetophytes, and lycophytes is not homologous (i.e., inherited from a common ancestor that produced secondary xylem), the genes that underlie the regulation of wood production by the axial polar auxin flow in the three clades probably are homologous (i.e., they probably are paralogues).

As a result of the extensive extinction that has occurred within all ancient clades of vascular plants (Mishler, 2000), including the large woody trees of lycophytes and equisetophytes investigated in this study, there is a rapidly growing recognition that the in-depth understanding of evolution at both the genetic/regulatory and organismal levels relies heavily on paleontological data. Information presented in this study reveals that such data are, indeed, available from fossil plants. Although the work presented here is restricted to increasing our understanding of growth of secondary vascular tissues in terms of regulatory mechanism evolution, the rapidly growing number of newly developed techniques for recognizing developmental regulatory mechanisms from fossils (Boyce & Knoll, 2002; Rothwell et al., 2006; Sanders et al., 2007) promises to increase dramatically the breadth of such studies. In so doing, paleontology is becoming more intimately incorporated into the mainstream of plant evolutionary studies.

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PHYLOGEOGRAPHY, FOSSILS, AND NORTHERN HEMISPHERE BIOGEOGRAPHY: THE ROLE OF PHYSIOLOGICAL UNIFORMITARIANISM[1]

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ABSTRACT

Biogeography is one of the most synthetic of biological undertakings; it requires placing a substantiated phylogenetic model in a geological, climatological, and ecological context, all of which shift through time. In the past two decades, the application of cladistic and molecular techniques has diversified our grasp on phylogeny. This has allowed the formulation of hypotheses of past distribution patterns based on samples from available living material and algorithms for their interpretation. Fossils contribute to these cladistic approaches by adding morphological checkpoints to character associations in time and by providing a basis for estimates of rates of divergence. However, fossils also check these hypotheses by direct occurrence (does a fossil of the taxon occur where predicted?) and by ecological suitability (is it reasonable that the taxon could occur in the predicted environment?). The first test is straightforward if difficult, requiring the finding of a specific fossil in a specific place and time. The second is based on the assumption of physiological uniformitarianism—that fossil and modern taxa united by a common morphology possess similar physiologies. If correct, then hypotheses of past distribution must accord with the predicted physiological tolerances of the taxon in question. Application of physiological uniformitarianism to phylogeographic hypotheses, together with new paleontological data, suggest (a) the validity of many established phylogeographic hypotheses; (b) the need to reevaluate others; and (c) the recognition that the North Atlantic land bridge likely functioned as a link between the Old and New Worlds into the Later Tertiary, contrary to this author's earlier papers.

Key words: Bering land bridge, boreal, fossil plants, North Atlantic land bridge, Northern Hemisphere, phylogeography, phytogeography, Tertiary, uniformitarianism.

The classic approach to the use of fossils in establishing historical biogeographic hypotheses was essentially scientific but not especially satisfactory. One would survey the known fossil record, assemble the reputable records of the taxon in question, and then suggest a pattern of migration consistent with their distribution in space and time. The resulting hypothesis was constrained by known deposits and existing identifications. This hypothesis could be predictive (it might suggest where new specimens could be expected to be found in time and space), but often the necessary fossil deposits to test the prediction were lacking. Furthermore, the discovery of a new representative of a given clade in an unpredicted time or place often required the hypothesis to be completely reconceptualized. Thus, advances took new data into account, but not infrequently left a historical trail of contradictory hypotheses. It would be far more satisfactory to narrow the possibilities through iterative testing and the refutation of a priori predictions (cf. Platt, 1964).

Recent advances in molecular techniques and data analysis provide a complementary approach to paleontology that helps to constrain phytogeographic hypotheses to a greater degree. Using molecular analyses of genetic material from living plants, coupled with analysis of morphological characters, practitioners invented the field of phylogeography (coined by Avise et al., 1987), overlaying phylogenetic interpretations on fossil and modern biogeographic patterns (e.g., Wen, 1999, 2001; Xiang et al., 2000; Donoghue et al., 2001; etc.). Furthermore, based on the gross assumptions of the molecular clock, phylogeographic hypotheses can also predict the time of phylogenetic splitting, placing a particular clade in a particular geographic locale at a particular geologic time, which creates a testable hypotheses based largely on non-fossil data. This is an important advance, as Donoghue et al. (2001: S41) note: "Geological and climatological processes that have impacted the biota of the Northern Hemisphere during the Tertiary are expected to yield little resolution when area cladograms are compared without taking the timing of diversification into account." Thus, phylogeographic approaches have the potential to offer alternative hypotheses to those generated from

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paleontological data alone. However, fossils retain an important role in this brave new world. First, the calibration of the clock for a particular clade is dependent on its antiquity as defined by the fossil record. This is a process that involves many assumptions and is fraught with complications that have been the focus of several presentations in this issue (Burnham, 2008; Gandolfo et al., 2008; Nixon, 2008). Additionally, Shaw and Small's (2005) caution on using molecular data from a single specimen, rather than exploring the potential for molecular variation within a species through multiple samples, deserves wider recognition. Second, fossils offer both a direct and an indirect method for testing phylogeographic hypotheses. Considerations of these tests and, particularly, the ecological fit of past distributions into phylogeographic models form the core of this paper.

Modes of Testing Phylogeographic Hypotheses with Fossils

Direct Testing—The Right Fossil in the Right Place

A phylogeographic hypothesis may suggest the presence of a particular clade at a particular geologic time in a particular geographic place. In general, the most important of these predictions will involve migrational bottlenecks. Thus, in the present case, the focus is on intercontinental disjunctions of taxa between the New and Old World in the Northern Hemisphere, in which the two greatest barriers are the Pacific and Atlantic oceans. These, in turn, create two bottlenecks over which terrestrial organisms need to pass—the Bering land bridge (BLB) and the North Atlantic land bridge (NALB). The former is still in existence, although limited by seasonal climate, while the latter has been affected both by oceanic widening (plate tectonics) and global cooling (Tiffney & Manchester, 2001).

The estimated time of the onset of genetic divergence logically should correspond to the origin of the interruption of gene flow. This interruption could result either from a migration event across a barrier causing the establishment of a new, isolated population or from the vicariance of a pre-existing and perhaps long-established distribution. These alternatives cannot be distinguished from molecular data, although a pre-existing fossil record can support the latter. However, both hypotheses imply the presence of representatives of one or both clades involved in the disjunction at the geographic locality that is inferred to form the barrier-to-gene flow at the time of its interruption.

If a phylogeographic hypothesis thus predicts the presence of a taxon in a particular place at a particular time, then paleontological data may be sought in the appropriately located and aged rock. For example, Donoghue et al. (2001) predicted that *Weigela* Thunb. and *Diervilla* Mill. (Caprifoliaceae) split ca. 5.2 ± 0.5 million years ago (Ma) to establish a present-day eastern Asian (*Weigela*)–eastern North America (*Diervilla*) distribution. The clade was hypothesized to have arisen in China or Japan and to have migrated over the BLB to North America.

At the time Donoghue et al. (2001) wrote, they were not aware of any fossils of *Weigela* or *Diervilla* in the Bering region. However, White et al. (1999), in a fairly obscure publication, place pollen similar to that of *Weigela* and *Diervilla* in northwestern Canada and Alaska, appearing 15 Ma and disappearing ca. 6.5 Ma. While the fossils suggest an older first appearance in the area and a slightly older disappearance than that suggested by phylogeography, the agreement is quite acceptable and the hypothesis is not refuted. However, it is to be noted that *Weigela* also occurs in the European Miocene and Pliocene (Mai & Walther, 1988; Mai, 2001) and that the hypothesis of a North American–European link must still be at least acknowledged.

Indirect Testing—Phylogenetic Uniformitarianism

Phylogeographic hypotheses place specific taxa in specific places at specific times. When they do so, the general ecological context of the surrounding vegetation should be in accord with the physiological tolerances of the living members of the clade in question. To use a trivial example, the Palmae are classically subtropical and tropical in distribution; a phylogeographic hypothesis that placed a palm in a temperate or boreal context would violate our understanding of the physiologic tolerances from modern examples.

This interpretation is based on the assumption of physiological uniformitarianism. Succinctly, we identify taxa in the fossil record by their morphology, assigning them to living families, genera, or occasionally species in the assumption that similar morphologies reflect similar underlying genotypes. However, the taxa in question also possess unique physiological characters that are again genetically controlled. If we make the identification of *Vitis* L. in the fossil record, we assign it to the genus on the basis of morphology and assume that it represents a liane, as *Vitis* is overwhelmingly dominated by vines (Mabberley, 1997). Similarly, we assume that it brings with it a set of physiological tolerances that allows it to grow in specific thermal and moisture regimes that fall within the tolerances of modern species within the genus. In the case of *Vitis*, this

results in a very broad range of ecological tolerance, as the genus extends from temperate to subtropical areas in the Northern Hemisphere (Tiffney & Barghoorn, 1976; Mabberley, 1997).

An alternative possibility must be considered: that morphology has remained sufficiently stable over time to allow placement of a fossil in a modern taxon while physiology has evolved independently, dissociating ecological tolerances from morphological identifications. If this were the case, flora and vegetation would exhibit a similar dissociation through time, and no clear relationship would be observed between cohorts of taxa and specific climates. While the nature of biological systems is too variable to state that this has never happened (e.g., that a genus of subtropical angiosperms evolved a temperate species to cross through a cooler climate, which, in turn, gave rise to a subtropical species upon re-encountering warm climates), the coherence of the history of vegetational and floral change through the Tertiary (e.g., Tiffney, 1985; Mai, 1995; Graham, 1999; Tiffney & Manchester, 2001), in concert with climatic change adduced from other sources (e.g., isotopic sources, Zachos et al., 2001), suggests that physiology is generally stable and tracks morphology.

To date, my discussion has implied that the physiological tolerances are those involving moisture and seasonal warmth. However, there are other aspects of physiology that potentially play an important role in determining clade survival in a given environment. Chief among these is the annual distribution of light at the poles. In previous papers (Tiffney, 1985, 1994, 2000), I have queried whether extended periods of polar winter darkness might prove to be a barrier in the Northern Hemisphere to the migration of evergreen taxa in periods of global warmth, as respiration would carry on in the absence of photosynthesis. If so, evergreen lineages might be restricted to more southerly migration routes in order to balance respiration with a minimum level of photosynthesis. This assumption appeared reasonable, given (a) the frequency of deciduous taxa in the fossil record of the North Pole (Falcon-Lang et al., 2004; Brentnall et al., 2005), (b) earlier experiments (Read & Francis, 1992), and (c) the importance of some deciduous taxa (e.g., *Larix* Mill.) in boreal realms in the present day. While this is a rational conclusion, in fact the current global climate is sufficiently cold that we do not have the opportunity to observe the response of thermophilic evergreen taxa to extended periods of darkness, as could have potentially occurred at times in the Paleogene of the Northern Hemisphere. Recent experiments suggest that the carbon loss involved in seasonal leaf shedding is far greater than the loss involved in dark-season respiration (Royer et al., 2003; Osborne et al., 2004) and that some feature other than carbon balance must drive the prevalence of deciduous taxa in the boreal realm. Brentnall et al. (2005) suggested that this feature reflected the frequency of fire-based disturbance. Such experiments are conducted over brief time spans; in order to survive and migrate, evergreen plants would have to succeed over a multigenerational time frame, allowing reproduction, dispersal, seedling germination, and establishment through repeated cycles. Further examination of both the role of carbon balance and/or fire seems in order, especially since another pattern of note is that many extant high-latitude austral taxa are evergreen. Other features may be involved in explaining the apparent barrier to evergreen taxa; these might be ecological (e.g., Givnish, 2002) or involve phylogenetic constraints inherent in the boreal taxa involved.

If we accept the reality of physiological uniformitarianism, then its application to testing phylogeographic hypotheses has one very useful aspect; it does not require the occurrence of the taxon under investigation in a particular place in the fossil record. As long as there is a fossil flora or fauna with sufficient detail to allow estimation of the paleoenvironment, or even a good geochemical estimate of paleotemperature, one can infer whether the particular taxon in question would logically have survived in the predicted environment.

However, this advantage is balanced by disadvantages. Foremost, it can only be applied to living taxa wherein the physiological tolerances of light, moisture, temperature, etc., are fully known. Furthermore, it cannot be effectively used on taxa with very broad tolerances. It would be difficult to support or falsify a historical hypothesis about a subtropical taxon if it were predicted that its occurrence fell within the scope of a warm-temperate vegetation. This aspect becomes more problematic in the earlier Tertiary where reduced climatic gradients allow the widespread occurrence of equable climes with interdigitated temperate and subtropical taxa. Nevertheless, the method still has power to refute hypotheses where the environmental tolerances of the taxon in question are substantially different from those of the predicted environment of occurrence.

Some Tests Employing Physiological Uniformitarianism

From the foregoing, we can proceed to look at a number of taxa for which phylogeographic hypotheses have been made, placing them in particular geographic situations and climates.

THE BERING BRIDGE

The BLB is a frequently invoked route of exchange between the Old and New Worlds in the Tertiary (Tiffney, 1985; Tiffney & Manchester, 2001). Several southern Alaskan Eocene floras hosted evergreen thermophilic lineages (Wolfe, 1972, 1977), which may indicate that the bridge was open to evergreen lineages at this time (see discussion in Tiffney & Manchester, 2001: S7). Later Eocene through Oligocene floras displayed an increasing dominance of warm-temperate to temperate deciduous taxa (Wolfe, 1972, 1992). By the Early to Middle Miocene, Alaskan floras hosted moderate- to cool-temperate taxa (e.g., Juglandaceae, Fagaceae, *Liquidambar* L., *Nyssa* L., *Tilia* L.; Leopold & Liu, 1994; White et al., 1997, 1999).

Many of the older crossings hypothesized from molecular evidence fit well within the physiological tolerances of the taxa involved. *Rhus* L. was suggested to be present on the BLB at ca. 34 Ma (Yi et al., 2004), *Menispermum* L. at ca. 29 Ma (Lee et al., 1996), *Campsis* Lour. at ca. 24.5 Ma (Wen & Jansen, 1995), *Gleditsia* L. and *Gymnocladus* Lam. at ca. 15–20 Ma (Schnabel et al., 2003), and *Liquidambar* L. at or before 15.6 Ma (Ickert-Bond & Wen, 2006). These are all warm-adapted, mesic taxa, and all but *Rhus* are deciduous; the latter includes deciduous and evergreen taxa.

Several more recent (Late Miocene–Pliocene) hypothesized crossings also fit within the predictions of physiological uniformitarianism. Thus, Nie et al. (2005) predict that *Kelloggia* Torr. ex Benth. (Rubiaceae) crossed the Bering Bridge ca. 5.5 Ma. Its distribution in montane western North America (viz. Burke Herbarium, 2006) and cool Asia fits this hypothesis. Nie et al. (2006a) predict that *Symplocarpus* Salisb. ex Nutt. and *Lysichiton* Schott (Araceae) crossed the Bering Bridge ca. 4–7 Ma. This, again, appears consonant with the Late Miocene–Pliocene Beringian vegetation and ecology of the extant taxa.

However, other hypotheses appear to pose problems for physiological uniformitarianism. Some are marginally problematic. Nie et al. (2006b: 1349) suggest that *Phryma* L. attained its eastern Asia–eastern North America distribution "at least 3.68 ± 2.25 to 5.23 ± 1.37 mya," or in the very latest Miocene through the Pleistocene. *Phryma* has a wide geographic range in North America, ranging from Manitoba to Florida, growing in rich and often moist woods (e.g., Fernald, 1970). Its geographic distribution in eastern Asia (Nie et al., 2006b: fig. 4) suggests an equally temperate environment in southeastern Asia north through Japan and Korea. None suggest an affinity for boreal forest conditions, which would have been present on the BLB and environs during the predicted time of crossing (Leopold & Liu, 1994; White et al., 1999). Nevertheless, *Phryma*'s wide modern distribution, especially in North America, offers the argument that it might be able to slip through the BLB during a brief warm spell within this time frame.

Other taxa are more clearly warm temperate in nature and are harder to imagine making the crossing in latest Miocene and Pliocene. For example, Xiang et al. (1998) proposed that *Calycanthus* L. (now occurring in China, western North America, and southeastern North America—a classic Tertiary refugial distribution, e.g., Li, 1952) crossed Beringia at ca. 3.1 Ma. Wen et al. (1996) and Zhou et al. (2006) place the crossing a little earlier at ca. 6 Ma. Similarly, Wen (1999, after Qiu et al., 1995) suggested that *Magnolia tripetala* (L.) L. split from *M. officinalis* var. *biloba* (Rehder & E . H. Wilson) Y. W. Law of China ca. 2–5.5 Ma. By 6 Ma, marginal exotics that had been present even two million years earlier (Reinink-Smith & Leopold, 2003, 2005) were largely absent from the coastal coniferous forests of Beringia (Wolfe, 1994). Post–6 Ma pollen records from central Alaska suggest a boreal forest (Leopold & Liu, 1994; White et al., 1999) with a few cool-temperate taxa (e.g., *Tilia*, *Corylus* L., *Juglans* L.). In light of its current locales of growth, *Calycanthus* would not be ecologically suited to these environments. While *M. tripetala* does co-occur with *Tilia*, *Corylus*, and *Juglans* in its present eastern North American distribution (Little, 2006), it has a more southern distribution than these other taxa. Additionally, the proposed crossing for *Magnolia* L. overlaps with the opening of the Bering Strait (Gladenkov et al., 2002). The lack of physiological consonance suggests that these phylogeographic hypotheses require re-examination. For example, while Schnabel and Wendel (1998) placed *Gleditsia* in Beringia at 3.9–4.7 Ma, again associated with an implausibly cool flora, Schnabel et al. (2003) re-evaluated this conclusion and placed this crossing in a much more appropriate climatic setting at 15–20 Ma.

While I focus on the bottleneck created by the BLB, in fact, the hypothesis of such a crossing involves more than just the transition across the BLB—it also invokes migration to the bridge and from it to the modern areas of distribution. Thus, a hypothesis involving the crossing of the BLB by *Magnolia tripetala* or *Calycanthus* also must account for the movement from the point of crossing to their present areas of survival in eastern North America, if the postulated time of molecular divergence measures a migrational event. To make this transition, these taxa need not only deal with the ecological setting of the

vegetation of Beringia at circa 6 Ma, they must also cross the Rocky Mountains and the interior grasslands before becoming established in the mixed deciduous forests of eastern North America. Given the changing nature of North American vegetation through the Tertiary (Graham, 1999) and the ecological affinities of *Magnolia* and *Calycanthus*, this would be more easily accomplished in the Eocene through Middle Miocene rather than later in the Tertiary.

An alternative hypothesis is that these taxa achieved their distribution at an earlier time, but that the molecular divergence measures the time of their vicariance. This eliminates the objection of the need to migrate through other potentially inhospitable environments, but still leaves the incongruity of *Magnolia* or *Calycanthus* co-associated with boreal taxa in Beringia at the proposed time of interruption of gene flow. This leads me to wonder if, in cases of vicariance or migration, the actual molecular divergence lags behind the time of cessation of gene flow.

THE NORTH ATLANTIC LAND BRIDGE

In Alaska and northeastern Siberia, there is a relatively good fossil record through the Tertiary (Baranova et al., 1989; Wolfe, 1994; Fradkina et al., 2005). Therefore, it is possible to both track ongoing exchange throughout the Tertiary and test phylogeographic hypotheses in this area against fairly numerous taxonomic and ecological data. This has allowed me to be rather black and white in approaching the role of fossils in evaluating molecularly derived phylogeographic hypotheses and focus on support or refutation. Hypotheses and tests lie at the core of science at its best, but often the scientific process is far more labile, resulting not in tests and refutations, but in an awkward upward spiral of understanding as new data modify our interpretation of preceding data. I turn now to just such a case involving the NALB, where I believe that molecular phylogeographic interpretations coupled with new paleobotanical data suggest a changing interpretation of the history of this important exchange corridor.

In earlier papers (Tiffney, 1985, 1994, 2000), I interpreted that the NALB was an important route for biogeographic exchange in the earlier Paleogene based on a range of fossil evidence. Its importance in the late Paleogene into the Miocene was difficult to assess by direct evidence, but plausible arguments for the migration of taxa have been made in the Oligocene (Hably et al., 2000) and Miocene (*Liquidambar*, Ickert-Bond & Wen, 2006). However, post Early Miocene, I was dubious of its significance in light of missing biological (fossil record) and geological data, both to the east and west of Greenland. Our paleontological and geological knowledge of the possible later Cenozoic links between western Greenland and eastern North America remains quite limited but is illuminated by more recent findings.

The fascinating Pliocene flora from Kap København in northernmost Greenland (Funder et al., 1985; Bennike, 1990; Bennike & Böcher, 1990) suggests a tundra vegetation bordering on taiga. To the south and west, Pliocene floras (Vincent, 1990; Matthews & Ovenden, 1990) and insect faunas (Elias et al., 2006) on Prince Patrick, Meighan, Ellesmere, and Banks islands of Arctic Canada suggest a mixed hardwood/conifer forest in the late Tertiary giving way to larch-dominated taiga and tundra by 2 Ma (Vincent, 1990) in some places, and pine-dominated taiga in others (Matthews & Ovenden, 1990), including *Sciadopitys* Siebold & Zucc. on Meighan Island. Precise interpretation of the timing of vegetational change is complicated by a stratigraphy that is still being resolved (Fyles, 1990). Climatic interpretations of entomological fossils (Elias & Matthews, 2002) suggest that the latest Miocene through Pliocene had a lower latitudinal temperature gradient than at present, allowing for the wider spread of the taiga in this period (Matthews et al., 2003).

It would be valuable to trace these transitions back to the Miocene, but only the rich flora from the Middle Miocene Mary Sachs Gravel on Banks Island exists to provide a norm for mid-Tertiary floras of Arctic eastern North America. Interestingly, this flora includes *Glyptostrobus* Endl., *Metasequoia* Hu & W. C. Cheng, *Juglans*, *Liriodendron* L., *Phyllanthus* L., and *Actinidia* Lindl., among other taxa (Matthews & Ovenden, 1990). No further Miocene floras are presently reported between the high Arctic and 35°–40°N latitude in eastern North America. The best continuous evidence for sub-boreal vegetational and climatic change throughout this time frame in the North Atlantic region comes from ca. 50°N latitude in Germany (Utescher et al., 2000).

Turning to geography, we presently lack evidence establishing or refuting possible physical links between western Greenland and eastern Canada south of the northern tip of Greenland in the mid-Tertiary. A second line of evidence for estimating the timing of the opening of the Banks Strait might involve tracking changing biogeographic affinities of marine invertebrates in these waters in response to the opening of the Bering Strait (e.g., Marincovich, 2000), although I am unaware that such data currently exist.

Since publication of my previous papers, several phylogeographic hypotheses have appeared that support later Tertiary exchange via the NALB. For example, Whitcher and Wen (2001: 296) suggested *Corylus* sect. *Corylus* migrated from Europe to North

America "in the late Pliocene or Pleistocene." Realizing both the purported physical barrier and real climatic barrier (taiga and tundra), they invoked long-distance dispersal, perhaps via a bird. This hypothesis requires a lot of the bird, given the distance, the prevailing westerlies, and the size of a *Corylus* fruit. Similarly, Milne (2004), using molecular data, suggested that *Rhododendron* L. subgen. *Hymenanthes* (Blume) K. Koch subsect. *Pontica* Tagg achieved a distribution between Europe and North America ca. 6 Ma or less. Given existing geological and paleontological data, I was predisposed to assume that both timings were anomalously young.

Quite recently, Denk et al. (2005) and Grímsson and Denk (2007) provided an updated evaluation of the Miocene floras of western Iceland, together with a discussion of their geologic setting. These authors placed *Corylus* on proto-Iceland between 12 and 8 Ma in an ecologically suitable temperate flora (vs. the suggested 3–2 Ma via molecular hypothesis) and in the same paper placed *Rhododendron* aff. *ponticum* L. in a 12–10 Ma Icelandic flora (vs. 6 Ma as suggested by Milne [2004]). Their geological analysis focused on the links between Greenland, Iceland, and Europe, and made clear that terrestrial bridges or island stepping-stone options were present through the period, diminishing toward 6 Ma. While their work does not address the Greenland–North America barrier, it clearly indicates the feasibility of a late Tertiary Europe–Greenland link.

In light of these data, the history of both *Corylus* and *Rhododendron* aff. *ponticum* would be consonant with their having achieved a European–North American distribution in the Middle to Late Miocene, followed by a vicariance event. But, as with the Bering example, the molecular data predict that the vicariance event occurred substantially later. Is there any evidence suggesting the possibility of a suitable environmental corridor in the Late Miocene through Pliocene?

Glaciers capable of generating extensive ice-rafted dropstones in the Norwegian–Greenland Sea existed in the Late Eocene (Eldrett et al., 2007), indicating an early development of a cooling climate on Greenland. Ice-rafted debris became more frequent in the mid-Miocene and gathered in strength through the Pliocene into the Pleistocene, but there is evidence to suggest that there was a significant variation in the strength of this process (Thiede et al., 1998). Thus, the ca. 2 Ma taiga-tundra vegetation at the northern point of Greenland (Bennike, 1990) suggests the possibility of a deciduous forest over more southern portions of Greenland in the latest Neogene. It is perhaps worth noting that a roughly 300,000-year warm period in the mid-Pliocene is associated with the re-invasion of Antarctica by *Nothofagus* Blume forests (Hill & Scriven, 1995; Haywood & Williams, 2005), suggesting the feasibility of rapid vegetational response to relatively short-term climatic fluctuations. Repenning (1990) provides an interesting discussion of the interplay of topography, climate, and vegetation in the Late Pliocene and early Pleistocene, hypothesizing short periods of warmth affecting eastern North America north to the eastern Canadian Arctic. While such events have not been clearly demonstrated in the later Miocene or Pliocene of the Arctic, their brief duration would make them difficult to detect, and they might have had an effect on the ability of deciduous taxa to migrate across Greenland as late as the Pliocene or earliest Pleistocene.

In sum, the fossils and the molecules point toward the need to reassess the possibility of a later Tertiary North Atlantic biogeographic link. The NALB may still have been functional in the later Neogene. However, we need more data, especially in Arctic eastern Canada and Greenland. The exposure of sediments with such evidence could become a positive side to current global warming.

One consistent observation that arises in comparing the paleontological and molecular data as presented here is that the fossils and the phylogeographic predictions converge but do not agree on the dates of several Neogene migrations. The molecular data seem to generally predict younger dates than the fossil occurrences or the application of physiological uniformitarianism would suggest. Are paleontologists simply missing data, particularly for short-term climatic fluctuations that open windows of opportunity for migration in the latest Tertiary? Or do most disjunctions arise from the vicariance of pre-existing distributions, as the fossils often suggest? If the latter mode dominates, the discrepancy in timing observed between the fossils and molecular predictions becomes a pattern worthy of investigation. Practitioners of both disciplines have to confer to see if a source of consistent error can be identified, again as discussed by several participants in this symposium.

Discussion

The theme of this symposium was paleobotany in a time of molecular genomics. This could easily be taken as an invitation for paleontologists to weigh the fossil record against the neontological perspectives and tools of molecular systematics. However, at the risk of oversimplification, the question is not the choice of fossils or molecules as has occasionally been propounded by discussants on both sides. Rather, the reality is fossils and molecules. Our two fields exist in a circumstance of reciprocal illumination. Fossils can

test predictions arising from molecular phylogenies, and molecular phylogenies can identify possible times and routes requiring further paleontological investigation. To achieve this, both sides must freely discuss their limitations and insights with practitioners of the other discipline.

But there is a further insight from this symposium, one I feel has not been properly realized. The central question is not fossils in an age of molecular phylogenetics but rather the role of desktop computing in both disciplines. This symposium is a testimony to the growth in power of molecular techniques in roughly the past two decades. But these techniques are informative only because of our ability to analyze the immense amount of data they generate using desktop computing. I suggest that the same set of tools will lie at the core of the successful integration of molecular and fossil data to understand the evolution of the flora (or fauna) of the last ca. 60 Ma. I believe we will truly advance only when all paleontological data are easily available online so that molecular phylogeneticists can check temporal calibrations and the goodness of ecological fit of their hypotheses—and this is but one use of such data. To this end, if paleobotany is to be a full partner in evolutionary biology in the age of information, then all verified paleobotanical data need to be databased in an internationally available, online system. Then we can use this summed data to seek and test patterns in evolution and distribution, and fully realize the interdisciplinary power of phylogeography.

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PLANT PALEOECOLOGY IN DEEP TIME[1]

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ABSTRACT

The paleoecology of plants as a modern discipline, distinct from traditional floristics or biostratigraphy, has undergone an enormous expansion in the past 20 years. In addition to baseline studies characterizing extinct plants and plant assemblages in terms of their growth habits, environmental preferences, and patterns of association, paleoecology has converged on neoecology and represents a means to extend our basic understanding of the world and to contribute to the theoretical framework of ecology, writ large. Reconstruction of whole plants, including studies of physiology and developmental biology, and analyses of biomechanics have become mainstays of autecological studies. Assemblage studies now are informed by sophisticated taphonomic models that have helped guide sampling strategies and helped with the interpretation of statistical data. Linkages of assemblage patterns in space and time with sedimentology, geochemical proxies for atmospheric composition and climate, paleosol analyses, and increasingly refined geochronological and sequence stratigraphic data have permitted paleoecologists to examine rates and extents of vegetational response to environmental change and to time intervals of quiescent climatic conditions. Studies of plant-animal interaction, explicit consideration of phylogenetic information in assessing assemblage time-space dynamics, and examination of ecological structure in terms of developing metabolic scaling theory are all having direct impact on paleoecological as well as neoecological studies. The growth of paleoecology shows no sign of diminishment—closer linkages with neoecology are needed.

Key words: Environmental biology, paleobotany, paleoecology, taphonomy.

The discipline of ecology encompasses the characterization of multispecies systems, the description of their spatial and temporal dynamics, and the search for organizing principles and general models. Of course, any explanatory models developed from studies of modern systems should apply consistently to long-extinct ecological realms as well, if they are to claim generality. Thus, in the largest theoretical sense, ecology not only should, but must, include and integrate data from modern systems with those from the past. The richness of empirical neoecological studies, which include more observable system-level dimensions than paleoecology (not the least of which is the possibility of experimental manipulations), nonetheless is limited temporally. Paleontology provides access to biological phenomena on a wide spectrum of time scales. But, in contrast to neoecology, it has limited spatial resolution at finely resolved time scales, is restricted to comparative data only, and includes many systems that are composed of organisms about which biological understanding may be limited. However, neobiology and paleontology overlap in many ways and together can be the basis for a robust understanding of the ways in which organisms respond and interact over short and long spans of time. Such responses may be either to environmental or biological crises of differing magnitudes, or both.

This paper is intended to review the broad field of plant paleoecology in the pre-Pleistocene, including the fundamental building blocks of the discipline, as well as considerations of the application of the basic data to problems of broader theoretical interest and importance. We do not believe that any caveat or apology is necessary for the various limitations presented by the fossil record. The matters at hand are: what can be done with the record; to what degree can its undoubted biases be understood, considered, and accounted for; how can one evaluate those data in real time to understand the ecological dynamics, preserved therein; and how can models based on extant systems be tested against that record? It is the intent of this contribution to provide a way into the literature of this rapidly growing, increasingly diverse field, while trying to identify the major areas of research, mature and just developing.

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Major Areas of Research

Paleoecology is a discipline that includes research approaches that range from morphology and floristics, which might be considered the traditional strengths of paleobotany, to some that were embryonic 25 years ago, such as growth modeling, evaluation of physiological proxies, and linkages between biotic and abiotic studies (geochemistry, sedimentology, paleopedology). The immediate questions of interest in each of these areas vary considerably. The following discussion addresses what seem to be the major foci of current research. Basic systematic investigations remain the core of paleobotanical research, and the continuance and importance of those endeavors are implicit throughout this paper. Alpha systematics provides the basic language with which we describe multispecies systems, and phylogenetic analyses are an increasingly important part of ecological studies.

AUTECOLOGY

The reconstruction of past organisms, as much as possible as whole plants, is probably the fundamental building block of paleobotanical research and, with a twist, of paleoecological studies. The key that takes this endeavor into ecology is the step from systematics as the end goal and the move into the ever-growing focus on functional morphology, growth architecture, and whole-plant reproductive biology, which ultimately aims at understanding autecology in as much detail as possible. The real challenge in this research is to see the past on its own terms. In other words, the challenge is to imagine and allow for the possibility that extinct organisms may have functioned in ways not known today, and that such function will, or can, be revealed in the gross morphology of the organism.

FLORISTICS AND VEGETATION STUDIES

Description of fossil floras, along with basic morphology, is perhaps the longest-standing research area of paleobotany. Development of protocols to describe floras quantitatively, to sample them in ways that permit reconstruction of original vegetation, and to analyze them statistically, is a major area of study and a building block of larger studies of ecological dynamics in time and space.

RESOLUTION

Considerable effort has been devoted to taphonomy—the study of the fossil record and the processes that underlie its origin. Perhaps the most fundamental paleoecological question in taphonomy is that of spatio-temporal resolution. Before the record can be used to address questions in ecology, sensu lato, it is necessary to consider the limitations of the record in candid detail.

DYNAMICS OF ECOSYSTEMS IN RESPONSE TO PERTURBATIONS

Paleontology may be able to contribute uniquely to ecology through the examination of multispecies assemblage dynamics over time intervals not accessible to neobiological analysis. The most publicly visible of these kinds of studies are those that examine biological responses to major environmental perturbations, such as catastrophic environmental change (brought on by such things as an asteroid impact or massive volcanism), major climate change (global to regional temperature increases or decreases linked to precipitation patterns), or linkages between biotic dynamics and changes in atmospheric composition. Presently, researchers are, or have been, focused on several time intervals of major extinction in Earth's history, and one can ask just how much can be generalized from any one of these. In other words, are there dependencies or contingencies unique to any system at any point in deep time that limit generalization from it alone? Is any one extinction interval unique, and thus, not of broader interest? Are there temporal trends suggesting the evolution of system-level responses through time?

ECOSYSTEM ASSEMBLY OVER SHORT AND LONG TIME SPANS

There has been a long-standing discussion in ecology about the existence of levels of organization above species populations, but can such systems even be circumscribed realistically? Is there the possibility of emergent properties in multispecies assemblages? Consider the early debate between adherents of the superorganism versus individualistic schools of Clements (1916) and Gleason (1926). A vast literature exists about the concept of equilibrium in ecological organization (e.g., MacArthur & Wilson, 1967), extending to the relationship between ecological stability and complexity (May, 1973), and systems ecology (Odom, 1983). Recent debate has centered on Hubbell's (2001) neutral theory of ecological organization, including such concepts as metacommunities (Holyoak et al., 2005) and assembly rules (Belyea & Lancaster, 1999; Weiher & Keddy, 1999). There also is increasing interest in the relationship between the historical-phylogenetic structure and the ecological architecture of assemblages of organisms (Shugart, 1997; Webb et al., 2002).

Paleontological data can be construed to indicate long periods of ecological persistence, even in the face of environmental fluctuations (e.g., Brett et al., 1996,

2007; DiMichele et al., 2004). And although not particularly couched in the terminology of assembly rules, it does appear that ecological systems underwent long periods of evolutionarily driven assembly, controlled by processes not unlike those proposed for very general rules of ecological assembly in space (compare Valentine [1980] with Belyea & Lancaster [1999]). If there are very general rules, to what extent are they taxon or assemblage specific?

More recently, intriguing work with great potential impact has been conducted on scaling relationships among plant-body sizes, metabolic rates, and ecological factors such as self-thinning of both populations and multispecific stands (e.g., Niklas & Enquist, 2001; Niklas et al., 2003), which may relate directly to the manner in which plants sort out resources within multispecies assemblages. Aspects of the first two topics have been investigated in various deep-time taxa (e.g., rhyniophytes and zosterophylls, cf. Roth-Nebelsick et al., 2000; Roth-Nebelsick, 2001; angiosperms, cf. Roth-Nebelsick et al., 2001) as well as in universal generalities (e.g., Roth-Nebelsick et al., 1994a, b), with the possibility of applying these results to assemblage-level communities over the Phanerozoic.

ARE THERE UNIQUE PATTERNS RECOGNIZABLE ONLY ON LONG TIME SCALES?

Are there kinds/types/classes of relationships between ecological and evolutionary dynamics that can be understood, or even simply recognized, only within the context of the geological-time rate spectrum (see below)? The recognition of such patterns will likely be an empirical outcome only of trends identified within the fossil record.

The Geological-Time Rate Spectrum

There is no single entity that can be characterized as Geological Time. Rather, the geological record preserves a wide spectrum of resolvable time intervals. Thus, the problem is one of time resolution. A major element of time resolution in the geological record is time averaging (see Behrensmeyer et al., 2000), which is more pronounced in the invertebrate and vertebrate fossil records than in that of plants.

The stratigraphic record includes plant-bearing beds of various attributes, each of which represents accumulation of debris within one original environment. Each genetic assemblage (the stratigraphic interval over which the assemblage is preserved) also can be considered to be a time layer of different irreducible temporal thicknesses. These may range from those preserved instantaneously (10^0-year deposits), such as forest floors buried under volcanic ashfalls (e.g., Wing et al., 1993), to those that are catchments of material accumulated over hundreds of years, such as tidal estuarine settings and packrat midden deposits (e.g., Mazon Creek, Pfefferkorn, 1979; Pearson & Betancourt, 2002), to many thousands of years, such as deposits of resistant material floated into marine depositional environments (London Clay, Reid & Chandler, 1933). The thickness of a rock unit is not related necessarily to the amount of time it encapsulates. Thus, the biological assemblage from a given deposit (bed or horizon) may have accumulated over some period of time that cannot be further subdivided, depending on the nature of the rock unit in which it is embedded. It may be temporally irreducible at the time scale over which the deposit accumulated, which is its level of depositional (or taphonomic) time averaging. Such deposits can be combined into meta-assemblages, each of which can encompass a hierarchy of time units of variable length. Such units can be described as artificially or analytically time averaged (Behrensmeyer & Hook, 1992). Consequently, any depositionally or analytically time-averaged fossil deposit can be analogized to a photographic snapshot of the fossil record but taken with variable shutter speed. These shutter speeds range from ultrafast, such as in situ burial of a forest-floor litter (e.g., Gastaldo et al., 2004b), to ultraslow, such as the diversity of Linnean families sampled globally and lumped into geological stage–level bins (Rees et al., 2002). In the latter case, we can imagine shadowy species moving in and out of the exposure, much like the ghosts of people in 19th-century long-exposure negatives. Thus, the geological record preserves, in natural and artificial packages, a broad spectrum of time resolution. These time packages can be combined in various ways and manipulated analytically and statistically, providing a very powerful tool rather than irresolvable obstruction.

Approaches to the Study of Paleoecology

At its most fundamental level, plant paleoecology is built on studies of individual plants and reconstructions of plant assemblages. In each case, there are various, often high degrees of uncertainty that must be overcome or accommodated for credible interpretations to be made. Taphonomy plays a role at all levels of inference in structuring interpretations and must be considered explicitly as a key element in all paleoecological analyses.

PALEOAUTECOLOGY

Reconstruction of whole plants. Comparative morphology has long been the core of paleobotanical

research. The ultimate goal of this research is to elaborate and understand the history of plant life on Earth. However, because of their modular construction and open, continuous manner of growth, whole plants are rarely preserved, instead tending to fall apart into a variety of organs. Putting these plants back together is a challenge. Not only is the objective to determine what isolated fossil organs go together but, where possible, to determine such things as the timing of reproduction relative to the plant's growth and life history or to variations in its physical environment and, more removed, the proportions of biomass allocated to reproduction versus vegetative growth, to the extent this can be estimated.

Perhaps the greatest biofantasy of whole-plant reconstruction is the misconception that organ attachment is the only way to ascertain organ association in an acceptable scientific manner. It is necessary to recognize that reconstructions of fossil plants are hypotheses, subject to test and revision. The more explicitly they are reconstructed and the more clearly the bases for reconstruction are made known, the more rigorously they can be evaluated by the addition of new information. First of all, attachment hardly provides certainty of unique association (e.g., the stigmarian root systems of Paleozoic arborescent lycopsids, which are found in attachment or association with many different species of several genera of stems belonging in different families; Bateman et al., 1992). More important is the degree to which this perspective denies the basic probabilistic nature of the reconstruction process and the fundamentally inductive, statistical nature of science. Likelihood is the main means by which the reconstruction of extinct plants must proceed if we are ever to have more than a handful of species confidently reassembled. The underlying means for such reconstructions include recurrent association at sample sites, association at sites with only one kind of each organ, anatomical and morphological similarities among dispersed organs, and similarities in the morphology and anatomy of organ cuticles and epidermal cell patterns.

The most important point may be that in paleoecological analyses, it is necessary to work with a variably complete understanding of the biology of the organisms, and keep hypotheses congruent with what is known or what can be reasonably inferred from the data. Some recent examples of the application of these various approaches to whole-plant reconstruction are highlighted below. There are a relatively large number of plants, too many to cover exhaustively here, that have been completely or partially reconstructed by various means, but these still represent just a tiny fraction of the plant species thought to have existed in the geological past.

Reconstruction from organ attachment is the most difficult of all kinds to undertake and requires attention to fine detail as well as a little luck. Perhaps the greatest abundance of whole plants is found among the early land plants of the Late Silurian and Early to Middle Devonian. Because of their simple morphologies, complete or nearly complete plants, including reproductive organs, often have been found and reconstructed. Such reconstructions have been effected directly and most often from compression fossils, some of which also have anatomical details preserved via permineralization. A few examples include the enigmatic fungus *Prototaxites* Dawson (Hueber, 2001; Boyce et al., 2007), the lycopsid *Sawdonia ornata* (Dawson) F. M. Hueber (Hueber, 1971), the trimerophyte *Pertica quadrifaria* Kasper & Andrews (Kasper & Andrews, 1972; Gensel & Andrews, 1984), and numerous plants from the classic Rhynie Chert in Scotland, which usually have been reconstructed by finding parts attached in series rather than as single whole plants (Paleobotanical Research Group, University of Münster, 2007). One of the most puzzling nearly whole plants is *Cooksonia* W. H. Lang, often touted as the earliest vascular land plant but known only from aerial parts (Edwards et al., 1992). Where were the prostrate axes to which these organs presumably were attached? Rothwell (1995) suggested that *Cooksonia* may have been a sporophyte incapable of a free-living existence, instead growing attached to a photosynthetic gametophyte, much like modern moss sporophytes and, thus, far from completely known morphologically. Gerrienne et al. (2006) found a cluster of Early Devonian *Cooksonia* axes attached to a thalloid-like pad and offered three possible interpretations, among which was the possibility that the axes represented sporophytes attached to a gametophyte. This hypothesis has been given biomechanical and physiological support by Boyce (2006), who demonstrated that most *Cooksonia* species are too narrow to have had sufficient photosynthetic tissues to sustain themselves independently.

The earliest fully reconstructed seed plant, *Elkinsia* Rothwell, Scheckler & Gillespie (Rothwell & Serbet, 1992; Serbet & Rothwell, 1992), is known to a degree that the timing and extent of reproductive allocation can be evaluated (e.g., Rothwell & Scheckler, 1985; Scheckler, 1986). Reconstructions are not limited to members of the understory; creeping ground cover and/or lianas are also are well represented (Manchester & Zavada, 1987; Krings & Kerp, 1999). The late Paleozoic gymnosperm *Callistophyton* Delevoryas (Rothwell, 1981) may be one of the most fully reconstructed plants of the entire geological record.

The detailed nature of Rothwell's (1981) reconstruction, based on permineralized anatomically preserved specimens, has permitted some revision based on findings from compression fossils (Galtier & Béthoux, 2002), illustrating the concept of reconstruction as testable hypothesis.

Whole-plant preservation is most likely for small plants. For example, the only known herbaceous conifer, *Aethophyllum* Brongn. from the Triassic, was reconstructed from a complete plant, a finding made perhaps more likely because of the small stature of the plant (Rothwell et al., 2000). Some other whole plants found in the fossil record include the isoetalean lycopsids *Clevelandodendron* Chitaley & Pigg (1996), of Late Devonian age, and *Chaloneria* Pigg & Rothwell (1983) and *Hartsellea* Gastaldo, Gibson & Blanton-Hooks (2006) of Pennsylvanian age, all with pole-like growth forms and comose base. The former is a single specimen terminating in a fertile zone, whereas *Chaloneria* was put together from a number of variably complete specimens, terminating either in a fertile zone or with intercalated fertile and sterile zones. *Hartsellea*, on the other hand, was preserved erect and in situ within a back-barrier marsh. Escapa and Cúneo (2005) reconstructed the early equisetophyte *Peltotheca furcata* Escapa & Cúneo from segments of plants that permitted linkage of reproductive and vegetative parts. Small aquatic plants occur in taphonomic settings that favor intact preservation. Examples include *Archaefructus liaoningensis* Sun, Dilcher, Zheng & Zhou, an aquatic angiosperm (Sun et al., 2002a), and *Hydropteris pinnata* Rothwell & Stockey (1994), a heterosperous aquatic fern, each proposed as the basis of new families of phylogenetic significance.

Larger shrubs and/or trees are extremely difficult to find preserved as whole plants. Rare discoveries of juveniles (e.g., Stidd & Phillips, 1968) can provide information about development and assist in the reconstruction of adult plants known only from isolated organs. The discovery of whole trees does occur occasionally, however. For example, Wnuk and Pfefferkorn (1984) discovered a forest of lycopsid trees with medullosan understory blown down during a storm and buried in place. The medullosans conformed to two growth habits. In one, trees were solitary, upright in growth stature, with trunks more than 20 cm in diameter and less than 5 m in height. Fronds were closely spaced, about 10 per meter of stem length, and were curved downward after death, suggesting development of a skirt, a morphological means of protection against epiphytes and vines. The other form was found in groups, suggestive of thickets in which the large fronds intertwined, allowing the trees to be mutually supporting. Stems were flexuose, up to 13 cm in diameter and greater than 5 m in height, with widely spaced fronds that appear to have been shed regularly rather than remaining attached. Pfefferkorn et al. (1984) evaluated published reconstructions of medullosans, which show a wide range of variation on the flexuose versus upright habit and determined that most were considerably inaccurate. Unfortunately, while revealing a great deal about medullosan growth habits, the actual foliage of these plants was not preserved attached; hence, taxonomic identity could not be established.

Partial reconstructions from attachment may sometimes have greater ecological significance than full reconstructions. Consider, for example, the Mesozoic cycadeoids, reconstructed primarily from silicified trunks, in which mature strobili were found at developmental states suggesting monocarpic (or "pseudomonocarpic") reproduction (Wieland, 1921). Similarly, the reconstruction of the false-trunk fern *Tempskya* Corda from the Cretaceous reveals a growth form unknown among ferns or seed plants today (Andrews & Kern, 1947). Fortuitous connection of stem/wood, sepals, and stamens with included pollen permitted the reconstruction of several plants by Manchester and colleagues (Manchester & Crane, 1983; Manchester et al., 1986; Manchester, 1989); we consider these to be "partial" reconstructions because the attached parts are from branches of presumed trees, and because the suite of organs found in attachment, although representing vegetative and reproductive parts, is an incomplete set.

Probabalistic means of assembling whole plants from dispersed organs, rather than through organ attachment, have produced a large number of reconstructions and given us considerable insight into the biologies of the plants. Paleozoic plants, in particular, are challenging because they generally have no close living relatives. Pennsylvanian arborescent lycopsid reconstructions, for example, historically illustrate egregious errors, particularly as related to the dynamic biology of the plants. Individually or in forest stands, these trees are almost always reconstructed along the lines of angiosperms. Trees are given light-capturing crowns and frequently are shown tipped over with their root bases in the air, presumably from blowdowns. However, evaluation of the development of these plants reveals dramatic architectural changes from juvenile to reproductively mature adult, which has major implications for individual plant and landscape reconstructions. Stands of these plants probably looked little like their representation in dioramas and paintings. Fossil data suggest two major growth forms, with different reproductive biologies. In one type, which is the most widely reconstructed, individual trees spent nearly all

of their lives as unbranched poles, forming a crown only at the end of tree life, which was determinate (Andrews & Murdy, 1958; Eggert, 1960). Anatomical (DiMichele, 1979) and compression (Wnuk & Pfefferkorn, 1987) studies indicate that cones were produced on these plants among the branches in the crown during the terminal phases of growth, rendering them effectively monocarpic (Bateman & DiMichele, 1991; Bateman, 1994). That cones were borne only in the terminal-phase crown suggests that height and tree crowns had little to do with light capture and much to do with dispersal of reproductive organs (DiMichele & Phillips, 1985; Phillips & DiMichele, 1992). The other growth architecture consisted of a determinate monopodial trunk bearing determinate deciduous lateral branches (DiMichele, 1980). Cones were borne among the lateral branches in some taxa or were borne directly on the trunk in others. In either case, the trees were polycarpic. The small, deciduous lateral branch systems were too small to account for much light capture in most species with this life strategy.

These peculiar trees had tenacious root systems that spread far from the trunk at shallow depths. The longest recorded stigmarian axis approaches 13 m. Neither of the authors have ever seen a tipped-up tree in their many visits to mines where stumps of these trees are exposed (e.g., Gastaldo, 1986; DiMichele & DeMaris, 1987; Wnuk & Pfefferkorn, 1987; DiMichele et al., 1996; Gastaldo et al., 2004a) nor have they read of any such tip-ups reported in the literature. Yet, uprooted lycopsids are staple elements of Carboniferous swamp reconstructions, implying that blowdowns and uprooting, for which there is no evidence, were parts of the disturbance mode in such systems.

Finally, anatomical studies of arborescent lycopsids raise certain matters with regard to their physiology (Phillips & DiMichele, 1992). The lack of a clear phloem connection between root and shoot, the generally limited phloem throughout the plant's aerial shoot, the leaf-like rootlets borne on the stigmarian axes, and the long leaves on stems and cones, are all consistent with extremely localized use of photosynthate and perhaps even self-supporting root systems in terms of carbon fixation. The purpose of this long discussion is to illustrate how much can be known and understood about fossil species from disconnected organs when intense work by many investigators is integrated into a series of reconstructions of considerable, though hardly absolute, certainty. This is even more remarkable given that a whole lepidodendrid lycopsid tree has never been found intact from top to bottom.

Disconnected organs can be linked into nearly living whole plants by using common features of gross morphology. An example of this comes from the work of Krings and colleagues. In a series of papers (summarized in Krings et al., 2003), they used cuticular features and gross morphology to link disconnected organs and reconstruct liana and vine-like habits in several species of Pennsylvanian-age pteridosperms. They detail a variety of climbing mechanisms, from hooks to pads to tendrils, that have structural analogues in taxa of other clades. In addition, they note much higher relative abundances and diversities of these plants in Late Pennsylvanian environments compared to environmentally similar Early and Middle Pennsylvanian tropical ecosystems. Clearly, there were major changes in the dynamics of these tropical forests, associated with major species turnover and change from lycopsid to tree fern canopy dominance, that favored the increase in vine abundance (possibly disturbance and patchiness of the forests; see Gastaldo et al., 2004b).

Strong patterns of organ association also allow whole plants to be reconstructed from disconnected organs, especially when an assemblage consists only of one of each organ type attributable to a particular higher-level group of plants. This is strengthened further by repeated occurrences of organ associations at multiple collecting sites. An example of this comes from the work of Kvaček et al. (2005), who proposed a reconstruction of a Late Cretaceous ginkgoalean consisting of the following dispersed organs: wood (*Ginkgoxylon greutii* Pons & Vozenin-Serra), short shoots (*Pecinovicladus kvacekii* Falcon-Lang), leaves (*Eretmophyllum obtusum* (Velen.) Kvaček), ovuliferous organs (*Nehvizdyella bipartita* Kvaček, Falcon-Lang & Dašková), and pollen (*Cycadopites* Wodehouse). They found this same association at four different collecting sites in consort with organs of other types, none of which were ginkgoalean in affinity. In addition, due to anatomical preservation of some remains, they were able to link the various organs sequentially in terms of their relative spatio-structural position on the plant. Finally, these plant remains occurred in a specific, recurrent lithofacies, indicative of peat deposits formed in salt marshes (making this the first described salt-tolerant mangrove ginkgophyte and indicating that the biology of this plant cannot be understood by analogy primarily with modern representatives, of which there is only one in the case of ginkgophytes). Other examples include *Archaeanthus linnenbergeri* Dilcher & Crane, an early angiosperm, reconstructed from partial attachment and associated organs, using anatomical similarities and the presence of distinctive resin bodies (Dilcher & Crane, 1984), and *Pentoxylon* Sahni, reconstructed from repeated associations of leaves, stems, and reproductive organs found in detailed sedimentolog-

ical context (Howe & Cantrill, 2001). Examples of organ linkage into whole or partial plants can be found in many groups and, remembering the hypothetical nature of reconstructions, is probably one of the strongest and most likely means of developing such associations.

The use of large samples from single sites also facilitates the reassembly of whole plants from isolated and partially preserved organs. An example of this is the work of Hernandez-Castillo on the early walchian conifer *Thucydia mahoningensis* Hernandez-Castillo, Rothwell & Mapes (Hernandez-Castillo et al., 2001, 2003). Hernandez-Castillo and colleagues were able to reconstruct this species, including its growth habit, from a large population of fragmentary compression fossils, some with preserved anatomy, including leafy branches, stems, and reproductive organs, and one specimen with branches attached to a stem segment. They concluded that early walchian conifers were considerably smaller than generally envisioned, perhaps reaching only 2 m in height, with architecture much like juveniles of the araucarian conifer *Araucaria heterophylla* (Salisb.) Franco (Norfolk Island Pine). Similar reconstructions of walchians, although of larger size, have been suggested by Ziegler et al. (2002a) based on much smaller populations of fossils. The larger-scale study informs and adds confidence to these other investigations and interpretations.

Whole-plant concepts can also be created by direct comparison with living descendants or close relatives. This is especially true of deciduous angiosperms, where isolated leaves make up nearly all of their fossil record, and post-Paleozoic conifers, where many groups have living descendants. It is also true of ferns, where most of the modern families appeared in the Mesozoic (Rothwell, 1994; Collinson, 2002; van Konijenburg-van Cittert, 2002a, b). Through a combination of morphologic and taphonomic/sedimentologic analyses and comparison with modern species, a complex of early Tertiary elms has been characterized and examined with regard to changing ecological preferences through time (Burnham, 1983, 1986). Taxa were found to have high degrees of ecological fidelity over millions of years. For conifers, consider the Eocene age mummified *Metasequoia* Hu & W. C. Cheng forests found north of the Arctic Circle in Canada (Williams et al., 2003a, b). Here, in situ stumps were found in association with mummified logs and thick beds of peat containing three-dimensionally preserved, but disconnected, cones and leaves in profusion, permitting reconstruction of plant biology and landscape structure. These plants can be compared directly to living *Metasequoia* from which a wide range of inferences is possible, including physiology (LePage et al., 2005). An example from Mesozoic ferns is that of Skog and Dilcher (1994) from the Cretaceous Dakota Formation. These deposits include species of the fern families Schizaeaceae, Matoniaceae, and Gleicheniaceae in wetland deposits, where they remained dominant even as angiosperms were increasing in other habitats. Today, members of these families form expansive tropical fern brakes in open areas or occur as undergrowth in more open forests. Direct evidence for this in the past is limited, but Skog and Dilcher (1994) infer this as a likely habit for the fossil taxa by analogy to these modern situations. They also propose that some Dicksoniaceae may have formed dense thickets, based solely on analogy to modern forms. In the absence of grasses, it has been suggested that ferns played a similar role, forming extensive areas of ground cover (LePage & Pfefferkorn, 2000).

Reproductive biology. The study of reproductive biology is a central part of both modern and fossil plant ecology. The most common approach, based on sheer numbers of published papers, has been the study of reproductive organs as isolated entities, generally placed within a particular evolutionary lineage or higher taxon but not tied to a particular whole-plant species (e.g., Taylor, 1982). Such studies have been compiled into analyses of patterns through time, most often with a focus on morphological evolutionary history (e.g., Taylor, 1965). Other studies have focused on functional morphology of reproductive organs, with a particular focus on seeds and pollination biology (e.g., Rothwell, 1972). Some of these studies have been experimental, using physical models, sometimes including mathematical modeling as a component (e.g., Niklas, 1981). A few analyses have looked at the distribution and relative abundances of reproductive versus vegetative organs of single species in attempts to estimate reproductive allocation and dispersal behaviors (e.g., Schwendemann et al., 2007).

The study of reproductive organs occupies too much of the literature to be detailed here. However, some compilations include Taylor (1978) on Paleozoic seed-fern pollen, Millay and Taylor (1979) and Rothwell and Eggert (1986) on Paleozoic seed-fern pollen organs, Tiffney (1986) and Sims (2000) on the distribution of seed sizes and dispersal syndromes through geologic time, Tiffney (1994, 1999) or Gee (2005) on seeds and fruits from Tertiary deposits, Crookall's (1976) summary of Late Carboniferous seeds, the massive compilation of seeds and fruits in the Eocene London Clay flora (Reid & Chandler, 1933; Collinson, 1984), and the compilation of late Paleozoic marattialean reproductive organs by Millay

(1979). In some instances, isolated reproductive organs can yield insights into likely pollination mechanisms, especially when comparisons can be made to extant relatives (Klavins et al., 2003).

Pennsylvanian arborescent lycopsid reproduction is understood in some detail. This is made possible by a wide range of studies of the structure and function of microsporangiate and megasporangiate cones (e.g., Brack-Hanes, 1978; Phillips, 1979; Brack-Hanes & Thomas, 1983; Pigg, 1983), linkages between microsporangiate cones and dispersed spores (which also permits differentiation of species from peat vs. clastic substrates [Willard, 1989a, b]), linkages between parent plants and reproductive organs (see Bateman et al., 1992, for summary), and morphological studies, as detailed above, that tied together growth architecture and reproductive patterns (DiMichele & Phillips, 1985; Phillips & DiMichele, 1992). Quantitative studies of coal balls (permineralized peat) from Pennsylvanian-age coals (e.g., Phillips et al., 1977; Phillips & DiMichele, 1981) have permitted coarse-grained studies of propagule distribution patterns; comparison of the distribution of certain seed-like megasporangiate units with vegetative parts of the parent plants (*Lepidocarpon* Williamson vs. *Lepidophloios* Sternberg) demonstrates that the propagules are dispersed more widely than vegetative material, consistent with distribution by flotation under aquatic swamp conditions and an invasive distribution strategy. Once more, this kind of analysis is made possible by a combination of detailed morphological analyses, whole-plant reconstructions based on likelihood of association, and study of statistical patterns of plant distribution in time and space.

The ecology of reproduction in ferns is facilitated by the common presence of spore-producing organs in intimate association with laminate, photosynthetic foliage. In addition, it is often possible to make direct comparisons between ancient and modern fern biology (Page, 2002) because many extant fern lineages were in existence during the Mesozoic, and, additionally, all ferns share certain reproductive and life-history traits. The late Paleozoic marattialean ferns, for example, were dominant elements of wetland ecosystems (Phillips et al., 1985) and are very well known morphologically, from both anatomical (coal-ball) studies and compression-impression fossil analyses. More cheaply constructed in terms of carbon allocation than any other group of Pennsylvanian wetland plants (Baker & DiMichele, 1997), with tree habit made possible by a mantle of adventitious roots filled with airspaces (Ehret & Phillips, 1977), these plants produced massive numbers of highly dispersed spores, permitting them to act as invasive weeds as well as forest canopy dominants (DiMichele & Phillips, 2002). Many of the basal extant families of filicalean ferns arose during the early Mesozoic (Tidwell & Ash, 1994; van Konijenburg-van Cittert, 2002a, b), their importance tapering off into the later Mesozoic and Cenozoic (Collinson, 2001; Wang, 2002). However, during the later Mesozoic, ferns remained ecologically dominant in many habitats and in many ecological roles, from colonizers of disturbed substrates to biomass dominants (e.g., Wing et al., 1993; van Konijenburg-van Cittert, 2002a, b). Cenozoic ferns are subordinate to angiosperms in most floras from this time interval, leading to a comparatively understudied record relative to ferns from older eras (see summary of Collinson, 2002), but are known to have occupied a range of habitats similar to extant ferns.

In some instances, it takes years to put together a picture of the biology of a fossil plant or group of plants. Reproductive biology is generally the most elusive part of such a reconstruction. A case in point is the biology of the Bennettitales, an important and diverse group of plants in many Mesozoic ecosystems that included both outcrossing and potentially inbreeding species. Members of the Cycadeoidaceae were considered to be monocarpic (Wieland, 1921) and likely animal, possibly beetle, pollinated (Crepet, 1974). In some species of the Williamsoniaceae, however, anatomical evidence supports wind pollination, seed dormancy, and outcrossing (Stockey & Rothwell, 2003).

Modeling studies of reproductive dynamics have been applied mainly to early land plants, where modern analogues of the fossil morphologies are lacking. Niklas (1981, 1983a) built scaled-up models of Late Devonian and Mississippian seeds and bombarded them with jumbo-sized, model pollen grains in wind tunnels. The seeds studied varied from those with completely unfused integuments and megasporangial modifications that facilitate pollen capture, described as hydrasperman reproduction (Rothwell, 1986), to those with closed integuments, some further enclosed in cupules. Such models allow direct testing of functional morphological scenarios. They also make specific predictions about how pollination biology of such ovules may have functioned, which can be tested against the fossil record (see comment and response from Rothwell & Taylor, 1982; Niklas, 1983b). Preliminary studies also have been carried out on pollen sedimentation rates, which have permitted testing of hypotheses about wind versus animal pollination in certain groups. For example, how do large medullosan pteridosperm pollen grains settle in liquids, such as pollen drops, relative to saccate pollen types, such as those

produced by early conifers (Schwendemann et al., 2007)?

Biomechanical attributes of fossil plants. Study of the biomechanical properties of plant tissues has permitted insights into growth and development that are difficult to attain by other means of reconstruction. Such approaches can be of great value even when only parts of plants can be evaluated (e.g., Speck, 1994). The grouping and distribution within the stem of anatomically preserved tissues can be analyzed and compared directly with modern analogues because of the physical nature of the system (Niklas, 1992).

Study of the growth architecture of the Pennsylvanian-age plant, *Sphenophyllum oblongifolium* (Germar & Kaulfuss) Unger by Galtier and Daviero (1999) illustrates a statistical approach to understanding plant growth form and its structural and ecological implications. By examining patterns of internode length, axis diameter, and leaf length, these authors were able to examine the growth ontogeny of the plant and infer scrambling, thicket-forming habit.

An example of contrasting approaches with convergent conclusions is demonstrated for the relatively well-known Middle Devonian aneurophytalean progymnosperm *Tetraxylopteris schmidtii* Beck (Beck, 1957), which was originally reconstructed as a small tree, perhaps as much as 3 m in height. Speck and Rowe (2003) studied the plant's anatomy in addition to growth architecture and concluded that it was not likely to have been self-supporting. In a more traditional but extremely detailed morphological analysis of a related species, *T. reposana* Hammond & Berry, the authors (2005) came to a similar conclusion regarding growth habit. *Tetraxylopteris* Beck is now considered to have been a small bushy plant that formed dense thickets of interlocking branches. Fertile branches were likely borne on the more distal parts of the plant, where the sporangia would be most exposed. The combination of such studies provides insight into this entire group of extinct plants and suggests that this kind of growth form may have been characteristic of the clade (Speck & Rowe, 2003).

Similar approaches have been used to examine growth form of the Calamopityaceae, an extinct group of Mississippian pteridosperms. These were small plants with limited secondary xylem, probably scrambling ground cover and/or vines. Rowe et al. (1993) analyzed their stem architecture and determined that internodal distances are relatively short in large-diameter stems but become increasingly long in smaller-diameter stems. The decrease is so dramatic that the small stems appear to have been incapable of self-support. Hence, it is possible that the mature plant had an upright or corm-like basal portion with a trailing or semi-self-supporting upper part. In contrast, Speck and Rowe (1994) conducted a biomechanical analysis of the much larger Mississippian pteridosperm *Pitus dayi* Gordon and concluded that it was certainly self-supporting and abscised its frond-like leaves.

The potential power of biomechanical studies is additive. In isolation, they may enhance understanding of the ecologies of individual plants. But, in aggregate, they allow those individual ecological insights to take on considerably greater significance. Most multi-taxon biomechanical and architectural studies, especially those that look broadly across clades, have been aimed at understanding evolutionary phenomena (e.g., Niklas, 1999). However, such studies can also be of fundamental importance in ecology. Consider the study of Niklas and Speck (2001), who investigated the evolution of structural safety factors to wind shear in early land plants, revealing distinct trends both through time and with regard to plant height, largely independent of clade membership. The phylogenetic independence of the trends is consistent with biomechanical properties of common structural elements and their stereotypical spatial deployment within plant stems. This approach opens the door to the estimation of wind effects in the context of its effect on stand density, plant morphology, and the relationships between wind, plant architecture, and reproductive strategies.

PALEOSYNECOLOGY—PUTTING TOGETHER A COMMUNITY

The reconstruction of past assemblages of plants is another of the central, and traditional, pursuits of terrestrial plant paleoecology. Until recently, such endeavors could be described broadly as floristics, the elaboration of a taxonomic list from one or more collecting sites in the same bed/sedimentological unit, or even from a time interval of variably long duration, with the underlying assumption being that they were growing in common, part of the same life assemblage. Often, visual or descriptive renderings of the inferred parent vegetation were made, generally representative of the species pool or even of the regional biome, although not explicitly stated or even recognized as such (e.g., Davies, 1929; Becker, 1972). The addition of sedimentological context to species lists greatly refines such studies, restricting the inferred assemblages to those taxa that grew in or proximate to the environment of deposition, thus making the list more representative of actual life communities (e.g., Hickey & Doyle, 1977; Scott, 1978; Wing, 1984; Spicer et al., 2002). The most representative reconstructions, both visual and statistical, are based on data, quantitative

and presence-absence, from field samples. The correlation of sedimentary settings with quantitative floristic composition, at as finely resolved a taxonomic level as possible, provides the closest approximation of the original vegetation. We will focus on such approaches. It should be evident from the following discussion that a thorough understanding of the sedimentologic, stratigraphic, and taphonomic framework is essential prior to any attempt to make paleoecological inferences from plant-fossil assemblages in space or time.

Taphonomic considerations. Vegetation and landscapes vary enormously in systematic composition, plant density, and vegetational architecture on continental spatial scales, influenced by climatic, topographic, and edaphic controls. Yet, plant remains are preserved only under a relatively strict sedimentological and geochemical subset of all possible conditions (Gastaldo, 1992a, 1994; Spicer, 1988, 1989). These conditions greatly reduce the likelihood of fossilization for many types of plant assemblages, depending on landscape position and climate. Additionally, there are a limited number of sites within any landscape where the potential exists to preserve plant parts because this can occur only in depositional regimes where: (1) dysoxia and/or anoxia prevails (i.e., at the sediment-water interface in a lake system); (2) micro-environmental geochemical gradients are strong (e.g., fluvial channel-bar troughs, Gastaldo et al., 1995); (3) resistant and diagnostic phytoclasts persist unaltered long after decay has removed all soft tissues (e.g., phytoliths, Strömberg, 2002; palynomorphs, Traverse, 1994); or (4) buried assemblages are maintained below the regional water table preventing oxidation and decay over the long term (10^3–10^8-year time scale; Gastaldo & Demko, 2005). In the majority of instances, biomass is fated to be recycled within the carbon cycle by the living biota—this is the rule rather than the exception.

The disarticulation of vegetative and reproductive structures, along with their tendency to degrade quickly, on the order of weeks (e.g., flowers and leaves), months (leaves), or years to several decades (wood, fruits, and seeds; Burnham, 1993b), presents some advantages and disadvantages when attempting to reconstruct anything, from a single plant to the original plant community or a landscape mosaic. It is especially rare for dispersal structures (e.g., seeds or spores) to be found attached to higher vascular plants. But, when such structures are found attached, it provides direct evidence for the operation of traumatic, as opposed to physiological processes, in the origin of the assemblage (Gastaldo, 1992a, 1994). It is well documented from neoecological and experimental taphonomic investigations that decay rates not only differ within taxa of a single clade or between various clades, but also under different climatic conditions (Gastaldo & Staub, 1999) and even within microhabitats under the same general climatic regime (Bray & Gorham, 1964). Additionally, the degree of similarity between a standing forest and the litter it produces may vary greatly and is not necessarily taxonomically proportional (including forest-floor litters; Burnham, 1989, 1997; Burnham et al., 1992), but depends on physiology and growth strategy (Gastaldo, 1992a). Yet, because decay rates of most plant parts are extremely rapid when compared with hard parts of invertebrates or vertebrates, it is possible to identify many of the constraining mechanisms and processes responsible for preservation of an assemblage. In addition, means have been developed to estimate the taxonomic diversity of the source vegetation (e.g., Burnham, 1993a; Gastaldo et al., 2004a). Hence, nearly all plant assemblages can provide what amounts to a T_0 estimate of the taxonomic composition of the source community and some degree of relative abundance of the source plants (this is more the case when leaves and other soft-tissue structures are preserved). It is true that certain resistant plant parts may be reworked, such as woody debris, charcoal, and palynomorphs, within particular depositional systems or particular intervals within stratigraphic sequences. Criteria have been developed, however, that allow for recognition of such recycled parts (e.g., wood-clast rounding, Gastaldo, 1994; change in palynomorph fluorescence, Traverse, 1988), the presence of which can thus be eliminated from consideration when evaluating an accumulation of plant parts from an identified transported assemblage.

There is an overrepresentation of wetland communities in the plant fossil record because these are in closest proximity to depositional regimes with the highest probability of preservation. Thus, such wetlands, particularly in the pre-Neogene, will account for the most fossils in autochthonous and parautochthonous assemblages. In situ, or autochthonous, assemblages are deposits of high importance and significance from which we can gain our greatest insights into community composition and spatial organization within the landscape mosaic. These assemblages consist of plant parts buried within the site where the source plants grew (Behrensmeyer & Hook, 1992). In general, there is little or no transportation of plant parts into or out of the area of burial, although there may be palynological contribution from outside the immediate area, especially if the source plants are wind pollinated. Hence, litters buried below the sediment-air interface, particularly below the mean water table, represent

those specimens capable of being preserved in the fossil record. Not all taxa contribute biomass equally to a litter assemblage. The contribution of any taxon may be a function of physiology, climate stress, or growth strategy, and the fidelity of the community within microhabitats of a landscape also may vary. For example, Burnham (1989: table IV, p. 16) found within a paratropical floodplain that the percentage of local species in the leaf litters, those within 100 m of the sample site, ranged from 45%–63%. The time at which litter is buried can further limit the fidelity of an assemblage, considering that different taxa contribute different proportions of their vegetative and/or reproductive structures during any growth year (Bray & Gorham, 1964), and that those proportions may vary seasonally and annually.

In particular depositional settings, such as within intermontane Tertiary sequences, care must be taken to differentiate the components actually preserved in situ from those that may have been transported downslope via various mechanisms, e.g., mudflows or lahars (Fritz, 1980a, b, 1981). Criteria have been developed to distinguish in situ from transported erect, root-laden trunks (Fritz, 1980b; Fritz & Harrison, 1985; Gastaldo, 1984, 1999). In coastal plains, such elements will be found in fluvial sediments and will have diagnostic features including root-encapsulated soil that may bear extrabasinal clasts (e.g., Liu & Gastaldo, 1992a; Gastaldo & Degges, 2007). Autochthonous burial can be instantaneous when entombment is the result of volcaniclastic ashfall (e.g., Burnham & Spicer, 1986; Wing et al., 1993), or over a period of days to months when increased sedimentation rate accompanies a change in local or regional base level (e.g., Ellis et al., 2003), which, in some instances, may be the result of coseismic subsidence (e.g., Gastaldo et al., 2004a).

Parautochthonous assemblages (Behrensmeyer et al., 2000) consist of disarticulated parts that have been transported some distance from the parental vegetation but not outside of the landscape setting in which they grew. Such assemblages most often are found within floodplain lakes (Rich, 1989; Gastaldo et al., 1989, 1998; Howe & Cantrill, 2001), but also in fluvial channel bedload and bar deposits depending on the fluvial dynamics and aerial extent of the vegetational unit across the landscape. In general, plant parts preserved in fluvio-lacustrine settings are derived from vegetation directly adjacent to and fringing the body of water. But in such environments, the potential exists for the assemblage to include allochthonous elements that have been transported greater distances, depending on the geographical and altitudinal position of the lake (e.g., Spicer & Wolfe, 1987) and the proximity of tributaries debouching into principal river channels (e.g., Gastaldo et al., 1987). Hence, it is imperative that an integrated geologic, taphonomic, and paleontologic approach be used to understand any assemblage prior to interpretation.

Allochthonous assemblages warrant caution when attempting to address certain paleoecological questions, although they may provide the only insight into the plants living in extrabasinal (Pfefferkorn, 1980) settings. In an intermontane lake deposit of Tertiary age (Taggart & Cross, 2000), it proved difficult to separate the plant parts originating in the fringing vegetation surrounding the lake from those transported into the lake from outside the area, particularly because all plant parts were transported as suspension-load debris. Use of phytoclasts as taphonomic features (e.g., bedload vs. suspension-load transported modification) in Gilbert-type delta–front deposits provided a means by which such local versus more regional contribution could be assessed (Spicer & Wolf, 1987). Using such data in conjunction with samples from other proximal and distal lake sites from the same geographic region, of the same approximate age, and formed under similar climatic conditions can permit assessment of the proportional contribution from different communities within the landscape to any given deposit. In assemblages preserved in offshore marine lowstand deposits (lowstand systems tract in the terminology of sequence stratigraphy), shore-fringing and more distal vegetation will be mixed and difficult to differentiate, thus limiting interpretation of the flora to regional spatial scales (e.g., Mapes, 1985; Rothwell et al., 1996). Often, the regional components of such floras are unknown in other parts of the stratigraphic sequence.

Sedimentary sequences, climate, and eustacy. The sedimentary context of a fossil plant assemblage is a crucial part of the equation that must be accounted for when using the record for paleoecological analyses. Often, these data are restricted to reports of the depositional environment (facies) and/or regional setting (correlative facies associations). But, without an analysis of the sedimentary sequence at a finer scale, it is possible to overlook pertinent information relevant to understanding the genesis of a plant-bearing deposit or rock sequence (Galloway & Hobday, 1996) or the interpretation of the fossil assemblage itself (Falcon-Lang & Bashforth, 2004; Gastaldo et al., 2005; Falcon-Lang, 2006).

Isotaphonomic assemblages (Behrensmeyer et al., 2000) must be distinguished and used when the paleoecological questions concern changes in taxonomic dominance or richness patterns in space or time. The identification of the sedimentary facies within a particular depositional regime, and the

taphonomic processes associated with that setting, provide the basis for comparison of communities. It is not sufficient to describe only the stratigraphic position or sediment matrix of the assemblage, because each of these criteria varies within a landscape. For example, Gastaldo et al. (1995) demonstrated that not all Carboniferous roof-shale floras preserved in coastal lowlands originate in the same way. Under specific depositional settings, these floras may be allochthonous, parautochthonous, or autochthonous, with the latter either preserving the ultimate peat-mire community (e.g., Gastaldo et al., 2004b; DiMichele et al., 2007) or a community that grew in a mineral substrate, unrelated to peat formation (e.g., Gastaldo, 1987; Falcon-Lang, 2006). The disparity in absolute time represented within such assemblages (e.g., coal-ball permineralized peats vs. buried-in-place clastic swamp vegetation) may be on the order of 10–100,000 years, depending on the combination of depositional and analytical time averaging. The same disparity holds true for floras preserved within the seatearth (paleosol) beneath a coal bed (Staub & Gastaldo, 2003).

The relative completeness of depositional sequences differs dramatically from continental to marine basins. Continental stratigraphies are the most incomplete, highly dependent on tectonic regime, basinal dynamics, and climate (Shanley & McCabe, 1994), with resolution varying within and between depositional environments (e.g., Pelletier & Turcotte, 1997; Lowenstein et al., 2003). Sediment transport from a source area is controlled by variations in long-term climate patterns, particularly the timing and amount of yearly rainfall (Cecil & DuLong, 2003). Similarly, rainfall patterns strongly control the distribution of plant communities. Thus, there must be a coincidence of plant preservation with periods of sediment transport, adequate physical accommodation space, and geochemical conditions that promote plant-part preservation rather than decay.

Although it may seem counterintuitive at first, little sediment is generated and transported in most environments wherever wet, humid to perhumid (rainfall 10–12 months/year; Cecil et al., 2003) climates prevail and precipitation exceeds evaporation and evapo-transpiration. Under these conditions, the landscape is covered by a multi-tiered vegetation. Extensive rooting at various levels within the soil profile binds sediments and prevents large-scale soil erosion and sediment transport down gradient to depositional environments. Pedogenesis (especially formation of podozols to laterites) is the principal process operating across the landscape under such climatic conditions (Parrish et al., 1993; Sellwood & Price, 1993). If soils contain sufficient proportions of expandable (swelling) clays, stagnant paludal conditions may occur with resultant peat accumulation (Gastaldo & Staub, 1995). Hence, records of plant communities that grew under these climatic conditions often include peat mires, with a lower probability for preservation of macrofloral remains in other parts of the landscape due to low sediment yield. At the other climate extreme, available sediment may be higher where evaporation greatly exceeds precipitation, but rainfall may be extremely limited and temporally concentrated (rainfall less than four months of the year in warm climates; Cecil & Dulong, 2003). Under such climatic conditions, however, sediment transport is restricted to times of high-intensity rainfall with high erosive power. Even with little vegetative cover to bind sediments, sediment transport is limited by the lack of water to do the work, which diminishes potential for organic matter burial and preservation (see the effect of water table below). Under such arid and semi-arid conditions, taxonomic diversity and plant density per unit area are relatively low. Plant material may be preserved in unique settings, such as alkaline lakes, which may preserve aerial debris from small deciduous taxa that surrounded the site (e.g., Beraldi-Campesi et al., 2006), or gypcrete and/or calcrete soils, in which subterranean rooting structures may be preserved. But, such occurrences are rare and may be associated with a rapid influx of sediment (e.g., volcaniclastic ashfall or higher-than-average rainfall resulting in fine clastic transport and deposition) and changing geochemical conditions. Hence, the probability of preserving plant material growing under extremely dry conditions is very low to insignificant, although such plant assemblages have been inferred from parts of the stratigraphic record (Retallack & Dilcher, 1988) and seasonally dry to arid communities have been reconstructed (e.g., Francis, 1984; Cantrill et al., 1995; Falcon-Lang & Scott, 2000; Falcon-Lang & Bashforth, 2004).

It would appear, then, that plant preservation in clastic rocks is promoted by equable climatic conditions, where precipitation equals or just exceeds evaporation and rainfall patterns are more seasonal in distribution (4–7 months/year), which would result in increased sediment load in streams and catchments (Cecil et al., 2003). The potential for plant-part preservation, though, still requires accommodation space for sediment burial and geochemical conditions that retard or prevent decomposition. Hence, it is more likely that a plant-fossil record will be generated within lacustrine, fluvial, tidal channel, and nearshore environments than in aerially exposed sites where pedogenesis and biomass recycling control the landscape (Gastaldo & Huc, 1992; Gastaldo, 2004).

In the broadest generalization, two sedimentological processes control the stratigraphic record in continental and marine environments, aggradational and degradational (erosional) processes, both of which are controlled by climate (Bull, 1991). As the concepts imply, aggradational sequences build up sediments in parts of a sedimentary basin, whereas degradational sequences remove sediments and redistribute them elsewhere within that or an adjacent basin. There is an interplay between both aggradation and degradation at various scales (within beds, architectural elements, channel-fill complexes, major depositional systems, etc.; Miall, 1990). Additionally, the record of each is influenced by autocyclic (e.g., channel migration) and allocyclic (e.g., tectonism, eustacy, climate) processes operating at any point in time and space (Beerbower, 1961, 1964; Demko & Gastaldo, 1996).

The highest probability for preservation of plant fossils exists within regional-scale aggradational sequences immediately above the contact with the previous regional-scale erosional event (Gastaldo, 2006). This point of transition between erosion and aggradation reflects a change in base level and the generation of increased accommodation space. Aggradational processes build up continental sequences via the transport and deposition of siliciclastics from an erosional source area. Sediment accumulation may occur in confined, intermontane basins within the source areas or further away, near local or regional base level. Pedogenesis may modify overbank sediments spread between the interfluves (Retallack, 2001), and soil-forming processes generally are assisted by the presence of some type of vegetational cover, the composition of which is controlled by the prevailing climate (Ziegler et al., 2003) and the invertebrate fauna that utilizes this resource (Raymond et al., 2001). The level of the regional water table will control the geochemical pore-water properties at all depths, concomitantly impacting decay of buried organic matter. Preservation of buried plant detritus is most likely when the regional water table is persistently high over millennial or longer time scales, rather than when the water table fluctuates dramatically, permitting percolation of oxygenated rainwaters through soil and buried sediments (Gastaldo & Demko, 2005).

Base-level changes in transitional (coastal plain, deltaic, estuarine, and tidal flat) and nearshore marine (delta front, barrier island) settings may either be tectonic or eustatic in nature, or a combination of both of these allocyclic mechanisms operating over different time scales. Debate continues as to how to tease apart tectonic from eustatic effects within the stratigraphic record as the primary mechanism controlling accommodation space for sediment burial (e.g., Klein, 1992; Christie-Blick & Driscoll, 1995; Miller et al., 1997). But, the fact remains that the long-term record of aggradational and degradational processes in coastal and marine depositional regimes may be controlled by eustacy (for this discussion, the short-term tectonic influences on generating accommodation and plant-part preservation will not be addressed; see Gastaldo et al., 2004a).

At certain scales of inquiry, paleoecology must consider the regional sedimentological framework within which ancient landscapes are preserved. The paradigm of sequence stratigraphy (Posamentier & Allen, 1999) provides a model that permits the effects of eustacy, climate, and tectonics to be integrated into a larger-scale framework. Eustatic cycles are based on the marine stratigraphic record and are asymmetrical over time, with rapid transgressive (onlap) sequences and prolonged regressive (offlap) sequences (Miall, 1990). Sequences are bounded above and below by unconformities, which often are exposed subaerial surfaces (identified by paleosols in terrestrial landscapes). Each onlap or offlap sequence is composed of parasequences, which are defined (van Wagoner et al., 1990) as a relatively conformable succession of genetically related beds bounded by marine flooding surfaces and their correlative updip (inland) surfaces (paleosols). Note that although the marine record may consist of a number of stacked parasequences, the continental record of these sea level changes may be locked within a single (thick) paleosol. Parasequence sedimentation occurs when accommodation is generated by a rapid rise or fall in sea level. Each change results in an asymmetrical, shallowing upward cycle somewhere within the basin but not everywhere coincidentally. Parasequences are stacked in various patterns across the basin over time. Transgressive systems tracts (TSTs) occur where successive parasequences overstep each other in a landward direction, and coastlines move over the continent, into more interior areas. When sea level has reached its maximum depth, the shoreline reverses its direction and progrades seaward, building up deposits of transitional and nearshore sediments in a series of parasequences within a highstand systems tract (HST). As sea level begins to fall, coastal progradation may continue until a time when the shoreline drops below the position of the previous shelf-slope break or one where the fall in relative sea level does change the position of the shoreline in the basin. The first situation results in the fluvial incisement (downcutting) and erosion of former HST deposits, and transport and deposition of these mobilized sediments as lowstand systems tract (LST) turbidites in deep marine settings.

For the general purposes of this discussion on transitional environments, preservation of autochthonous and parautochthonous fossil plant assemblages occurs primarily when sea level is high, that is, within aggradational HST parasequences. It is in these settings where high sedimentation rates and high regional water tables prevail (characterized by inceptisol formation and wetland vegetation). When sea level drops and LST deposition takes place, degradational processes operate within the then-exposed HST deposits. All buried assemblages are subject to effects brought on by a lowering of regional water table coincident with the newly established, lower (oceanic) base level.

In summary, climate not only controls the distribution of vegetation across the landscape but also the timing and nature of sedimentation, within which plant parts are buried and potentially preserved. For these reasons, the plant fossil record in more proximal and, therefore, somewhat higher-elevation siliciclastic continental environments is biased toward low diversity, seasonal to humid, wetland communities in which geochemical conditions exist that may retard decay of plant debris. When these settings are subjected to sudden base-level change (e.g., tectonic coseismic subsidence), new accommodation space is generated and filled rapidly. This sedimentary infilling (aggradation) is accompanied by a rise in regional water table, removing some buried organic matter from the effects of oxygenated groundwater. If climate remains relatively humid during landscape stasis, groundwater level will remain high and buried organic matter can be preserved. But, if climate trends toward more seasonal and/or semi-arid to arid during landscape stasis, water tables will drop and decay of buried organics will occur. Entombed organics may be lost entirely from buried sedimentary sequences if sediments are not somewhat compacted, or may be reduced to impressions where some compaction has taken place in fine-grained sediments. Early diagenesis may promote mineralization of buried detritus, including pyritization, limonitization, and permineralization with calcite or silica.

Plant assemblages in coastal plain and transitional settings are also biased toward wetland communities growing under seasonal to humid climates. The reasons are similar to those provided above. Coastal and transitional communities already are at or within ca. 2 m of mean sea (base) level and grow within a progradational regime where sediments, in general, accumulate. Even in these basinal settings, preservation potential is high only when accommodation space for sediments is generated either by localized tectonic or compactional subsidence and concomitant sea-level rise, or eustatic rise in association with transgression without ravinement (Liu & Gastaldo, 1992b). The latter situation commonly occurs within incised valleys where gradual sediment aggradation in a more proximal area, closer to the sediment source, allows for a thick sedimentary package in which plant fossils are preserved (Demko et al., 1998). Preservational potential is highest in subsequent parasequences during the phase of rapid aggradation, again, if the regional water table ascends as sediment accumulates and is maintained over the long term (fourth-to-third order cycle durations of 10^4–10^5 years). The fact that there is a bias toward an overrepresentation of wetland communities through geologic time, preserved within predictable settings by identifiable mechanisms, allows for their direct comparison under broadly isotaphonomic conditions.

Paleosols, biochemical, and geochemical proxies. Although now readily recognized and widely studied, it is hard to believe that until 25 years ago, paleosols were barely recognized in pre-Quaternary rocks. Paleopedology focuses on the soils that formed in past landscapes. Paleosol classification schemes may be based on modern soil-forming processes (Mack, 1997; Retallack, 2001b) or pedogenic processes that result in identifiable characters (Mack et al., 1993; James et al., 1998). The mosaic of soil types that exists across any landscape reflects the variation in physical and chemical features resulting from variations in climate and vegetation (Kraus, 1999). Soils may form in as little as a few hundred years (wetland entisol/inceptisol) or over several millennia (oxisol/spodosol), and well-developed soils usually define major unconformities in terrestrial stratigraphic sequences (e.g., Driese et al., 1994; Shanley & McCabe, 1994; Joeckel, 1995; Olszewski, 1996; Cecil et al., 2003; Demko et al., 2004). Evidence of colonization by plants and animals may include the presence of root traces or drab halos, where organic matter has been degraded and color differentiation enhanced by dehydration of ferric oxyhydrates during burial (Pfefferkorn & Fuchs, 1991; Retallack, 2001b), or ichnology (e.g., Hasiotis, 2003). Most often, though, organic matter is recycled through the soil system, leaving little or no physical evidence of the specific kinds of plants that biologically modified the sediment. Dispersed organics may remain within the soil profile, such as phytoliths (Strömberg, 2004), carpoliths (Thomasson, 1983), and meso- or microdebris; some may be identifiable systematically, whereas others may be amorphous and unidentifiable. Regardless, many plant parts retain original biochemical information (Jahren et al., 2004b, 2006) that can be used for paleoecological restoration. In addition, such debris may act as the nucleating agent

for soil nodules or concretions (e.g., Pye et al., 1990), if bacterial activity and geochemical conditions permit.

By their nature, rooted-in-place, autochthonous assemblages allow correlation between paleosols and the plant community under which they developed. But, because in situ assemblages are a rarity, the source plants and vegetation under which the majority of paleosols formed remain unknown. In addition, long-exposed terrestrial surfaces can be subject to changing climatic and biotic agents resulting in compound soils affected by a number of potentially very different kinds of physical and biotic conditions. The question further arises as to possible relationships between specific soil types and specific kinds of vegetation (arborescent vs. herbaceous; gymnospermous vs. spore-producing), and whether diversification of vegetation types over the Phanerozoic has resulted in the diversification of soil types. Such questions are focused more on those soils that form under seasonally dry conditions, such as vertisols, alfisols, and spodosols (Cecil & DuLong, 2003; Driese et al., 2005). In contrast, very wet and very dry soils, at the ends of the climatic spectrum, present plants with a unique set of physical and physico-chemical conditions controlled by persistently high water tables (e.g., histosols, laterites), or high evapo-transpiration rates and very low rainfall (e.g., calcisols, gypsisols) that affect vegetational structure independently of its phylogenetic composition. Note the great similarities among Late Mississippian, Pennsylvanian and Permian, Late Cretaceous, and Neogene peat-accumulating histosols, despite the enormous differences in the phylogenetic composition of the vegetation under which they formed (it is not possible to compare the other end of the spectrum because of taphonomic constraints).

There is evidence of change in soil structure with the advent of arborescent (tree and shrub) growth forms, accompanied by the increasing depth of root penetration (Driese et al., 2000). During the Early to Middle Devonian (Elick et al., 1998), soil thicknesses and types were controlled more by illuviation and translocation (Driese & Foreman, 1992; Driese et al., 1992) than by organic degradation and soil respiration. Pre-vascular plant-soil types have been described (e.g., polsterland; Retallack, 1992), presumably formed under a bryophytic vegetation (Strother, 2000). Many of the Middle Devonian soils in which autochthonous assemblages are preserved are poorly developed wetland paleosols (e.g., Allen & Gastaldo, 2006; Greb et al., 2006), although Retallack (1997) infers better-drained and -forested alfisols from Antarctica during this time (but see Dahms et al., 1998). A wider range of soil types appears within continental sequences during the expansion of gallery forests in the Late Devonian (Algeo & Scheckler, 1998). The subterranean plant parts preserved in these soils, if any (Pfefferkorn & Fuchs, 1991), are generally root traces, such as clay-filled voids (Retallack, 1999), rhizoliths (e.g., Schneider & Rossler, 1996; Kraus & Hasiotis, 2006), or rhizotubules and rhizocretions (Demko et al., 2004). Typically, the community structure responsible for origin of a paleosol is inferred by analogy with modern biomes (Retallack, 1992, 2001b). Such an approach is more consistent with angiosperm-dominated landscapes of the Tertiary (e.g., Retallack et al., 2001) than with ecosystems dominated by extinct gymnosperms and lower vascular plants.

Paleosol bulk-organic matter (OM) retains the carbon isotope ($\delta^{13}C$) signature of the contributing plant matter (Jobbágy & Jackson, 2000). Consequently, isotopes have been used as a proxy to recognize unique metabolic pathways (C_3 or C_4 grasslands; e.g., Fox & Koch, 2003, 2004; Pagani et al., 1999) and to characterize their relative proportions in parent vegetation. Isotopes also have been used to assess paleoclimate (e.g., Boom et al., 2002). Soil OM is derived from the decay of biomass produced under C_3, C_4, or Crassulacean acid metabolism (CAM) photosynthetic pathways. The C_3 and C_4 isotopic composition of carbon varies from −25‰ to −32‰ and −14‰ to −10‰, respectively. Presently, there is no evidence that C_4 pathways existed prior to the Miocene, although inferences have been made about CAM (Raven & Spicer, 1996). This fractionation persists upward through the food chain, with incorporation of the isotopic signal into tooth enamel (e.g., Fox & Koch, 2003, 2004). However, the carbon isotopic signal in soils may be altered by decay processes (e.g., Bowen & Beerling, 2004), leading to the use of taxon-specific biomarkers (e.g., Lockheart et al., 1997) that, in the absence of recoverable fossil-plant debris, may make it possible to differentiate the contribution of specific taxa (Nguyen Tu et al., 2004; Smith & White, 2004).

Stable carbon and oxygen isotopes recovered from calcite cements within soil carbonates (concretions, nodules, amorphous masses) also provide paleobarometric data for the time at which calcite precipitated (Cerling, 1984, 1991). Pedogenic carbonates have been used as an indirect method to estimate the paleoatmospheric CO_2 concentrations and climate, despite complexities for which analytic compensation must be made (Ehleringer & Cerling, 2002; Tabor et al., 2007), and several models have been developed (Cerling, 1991; Yapp & Poths, 1996).

In the cases where pedogenic carbonates reflect the incorporation of atmospheric CO_2, it is possible to

reconstruct paleoclimates and, in turn, paleoprecipitation regimes (e.g., Tabor & Montañez, 2002). There are numerous Tertiary-age examples based on multiple proxies (pedogenic carbonates and tooth enamel; e.g., Koch et al., 1995, 2003), with fewer further back in deeper time. Triassic climate, for example, generally has been interpreted as warm to semi-arid under monsoonal seasonality (e.g., Rees et al., 2002). Yet, Triassic macrofloral assemblages are interpreted to indicate the prevalence of warm, humid climates (Ash, 1999), probably reflecting the deposition of plant-bearing beds during wetter intervals within incised valley systems (Demko et al., 1998). Recently, Prochnow et al. (2006) acquired isotopic data from Triassic pedogenic carbonates. They estimated atmospheric CO_2 concentrations from the micritic fraction using the equations of Cerling (1999), and temperature using both oxygen and carbon isotopic values. They inferred a trend in the Early Triassic of the southwestern U.S.A. from arid to semi-arid (aridisols) conditions in the upper Moenkopi Formation to a more humid climate (alfisols) in the lower Chinle Formation, followed by a drying trend in the upper Chinle (semiarid aridisols and inceptisols). In this same lithologic sequence, there is an overall paucity of fossil plants, and those that do exist are concentrated within a pluvial interval, which is no surprise, given the taphonomic constraints on plant preservation (see earlier discussion). Hence, in those parts of the stratigraphic record where aridity and/or high seasonality is inferred from isotopic evidence, the absence of well-preserved megafloral assemblages is not unexpected, given the geochemical conditions.

The most comprehensive spatio-temporal insight into a fossilized terrestrial plant community comes from the integration of paleosol and (bio)geochemical data from rocks bearing autochthonous mega- and microfloral plant fossils (e.g., Gastaldo et al., 1998). It is most common, however, to encounter parautochthonous assemblages, which occur in association with, but not as part of, paleosols. Such assemblages may have little or nothing in temporal context with the paleosol(s) immediately beneath or above them within the section (Gastaldo et al., 1995b). Caution is advised particularly where there are incongruencies in paleoclimate indicators in successive beds, such as when megafloral assemblages occur in sequences with, but not actually within, calcisols or aridisols. Such paleosols form at that end of the climate spectrum where preservation of labile plant tissues is unlikely. Hence, only rarely can the presence of a well-preserved plant-fossil assemblage in such a rock sequence be used to interpret a dry or arid community.

Spatial patterns. Spatial relationships of taxa within the landscape are dictated by a variety of abiotic factors including edaphic (soil) conditions and topography, insolation, local water availability, and climate. Most landscapes are vegetational mosaics with taxa capable of growing anywhere conditions do not exceed the limits of their physiological tolerances (Beleya & Lancaster, 1999; Jackson, 2000). It is recognized that incumbency, competition, the role of light breaks, and other factors operate over the short term (10^1–10^2 years) to dictate the composition of any local community. But, in theory (e.g., Hubbell, 2001), over the longer term, all taxa within a species pool could be considered to be roughly equivalent and capable of growing anywhere within the region of the species pool or metacommunity (Law & Leibold, 2005), given the proper abiotic conditions. Hence, the composition of a plant-fossil deposit will depend on whether plant parts were shed physiologically (resulting in a lower fidelity record) or traumatically (resulting in a higher fidelity record), which plant parts were available at the time the deposit was formed, the chemistry of the depositional environment at the time, and the process-geometry of the sedimentary environment.

As a result of these factors, it is unlikely that most taxa in the immediate source vegetation will be represented within any given assemblage of fossil plants. Add to this fact the spatial scales of depositional environments, which are often orders of magnitude greater than what is exposed at most outcrops. It thus becomes clear that collections from a single hole-in-the-ground locality, less than 1 m^2 to several square meters, capture only a fraction of the forms that existed.

On the other hand, one must consider the regional metacommunity, broadly speaking, the regional species pool (Holyoak et al., 2005), that will contain many more species than any local site. This allows a solution to the limitations of small local samples, which is to make many small collections from across a sedimentary environment (e.g., Johnson & Ellis, 2002; Gastaldo et al., 2004b; DiMichele et al., 2007) or coeval stratigraphic horizon (e.g., Davies-Vollum & Wing, 1998). Statistically, such an approach will maximize the available information (Bennington, 2003), particularly where an autochthonous assemblage is sampled. This approach can be confounded by difficulties in tracing short-term time horizons over large spatial areas—the more finely one resolves time at a single outcrop exposure, the more difficult it can be to trace that time line laterally, without a well-defined and sedimentologically understood marker (such as an ashfall tuff). In addition, different times in geological history appear to have had different

landscape diversities, i.e., different metacommunity/species pool richnesses, even while retaining similar diversities on small, subhectare scales (Wing & DiMichele, 1995). This may involve a systematic time bias, such that Paleozoic species pools may be significantly less diverse than those of the Mesozoic and Cenozoic, leading to more uniformity among the best sampled sites along a gradient.

Sampling strategies. It is apparent that many interacting factors affect the genesis and maintenance of a fossil plant assemblage. In order to ensure maximum data recovery for paleoecological analyses, appropriate sampling strategies must be employed, dependent on the taphonomic signature of the assemblage, aerial extent of the exposure, both the lateral face and horizontal bedding surfaces, and degree of matrix lithification. Different techniques, for example, will need to be employed for autochthonous assemblages in well-lithified sediments, where standing vegetation and forest-floor litter are preserved in lithified mudstone (e.g., Taylor et al., 1992) versus those preserved in a non-lithified sediment in a semi-mummified state (e.g., Mosbrugger et al., 1994; Williams et al., 2003a, b). Similarly, lithified sediments preclude sieving and, thus, reduce the possibility of recovering small fruit and seed remains from parautochthonous assemblages, remains that would provide a more complete representation of the community than leaf or wood analyses alone (e.g., Gastaldo et al., 1998).

What's the question? Most macrofossil plant assemblages targeted for paleoecological analyses can be characterized broadly as parautochthonous. It is in these kinds of assemblages that the widest range of sampling strategies has been used. It is intuitive that the methods of sampling and analysis should fit the nature of the question (Magurran, 1998). One of the most significant questions regards the matter of biodiversity. One can ask legitimately what generates biodiversity, what does taxonomic richness reveal about ecosystem attributes and functions, and to what extent do rare species matter in comparative ecology? These have become subjects of considerable discussion in neoecology (e.g., Loreau et al., 2001; Ives & Carpenter, 2007). For example, Buzas and Culver (1999) demonstrated, using highly diverse assemblages of modern benthic foraminifera, that interregional differences (between the arctic and tropics, for example) in species richness can be accounted for mainly by the rare taxa that lie in the tails of species abundance curves, largely those known only once within huge numbers of samples (81% of the difference), very few of which have left a fossil record. Conversely, the top 10 dominant species in an area (species pool) were found to have approximately similar distributions and relative abundances in each biogeographic region. Thus, if one is interested in ecological structure, it is likely that the dominant species in a local area, such as a hectare, will emerge quickly from small samples and most certainly if multiple samples are taken (e.g., Burnham, 1993b; Wing & DiMichele, 1995).

If comparisons are to be made across allochthonous assemblages, presence/absence data collection is most appropriate. If the objective is to determine richness (α diversity) of a local assemblage, for example, the focus should be on large numbers of specimens from within a given depositional environment, in order to maximize recovery of taxa; it is not necessary to laboriously collect data on debris cover (e.g., biomass estimate based on digitized area; Pryor & Gastaldo, 2000). Similarly, if the question concerns the proportionality of taxa with the intent of reconstructing community structure, which is the question of most paleoecological studies, it is generally appropriate to use a quantitative sampling method, the choice of which will depend on the taphonomy of the plant deposit, the nature of the plant fossils, and the nature of the geological exposure. In such studies, the objective most often is to understand vegetational structure, so that exhaustive sampling to detect a high proportion of the rare species is likely not necessary nor would the results justify the sampling effort required.

Quantitative sampling. Several different approaches to quantitative sampling have been employed in paleoecological studies of fossil plant deposits. (1) In studies of angiosperm-dominated floras preserved in fluvial and lacustrine settings, it is common practice to count leaves individually (Wing, 1984; Johnson, 1992; Burnham et al., 1992; Burnham, 1994; Gemmil & Johnson, 1997; Davies-Vollum & Wing, 1998; Wilf et al., 1998a), permitting direct conversion of leaf counts to biomass estimates, if desired (assuming size and leaf thickness to be approximately the same across taxa of a given life habit). (2) In permineralized peats (coal balls) of Pennsylvanian-age coal beds, square-centimeter (Phillips et al., 1977) or square-millimeter (Pryor, 1996) grids have been used to estimate taxonomic and organ numbers and biomass when overlaid on cut surfaces. When extensive bedding surfaces are accessible, (3) point count (Scott, 1977a, 1978, 1985) or (4) quadrat methods (Gastaldo et al., 2004b; DiMichele et al., 2007) have been employed. (5) For mine spoils or museum collections from a single depositional setting, rapid estimates of relative abundance can be made by the use of hand samples, treated as quadrats (Pfefferkorn et al. 1975; DiMichele

et al., 1991). These methods produce similar but not always identical results when applied to the same data sets. Wing and DiMichele (1995), for example, compared quadrat (frequency) methods and percentage representation (count) methods and found that the quadrat method systematically increases the abundance of rare taxa while reducing the abundance of common taxa.

Proportionality of taxa within an autochthonous or parautochthonous assemblage also can be assessed using subjective and/or semi-quantitative measures. Simple rank-order abundance (ordinal scale measure) is an attempt to assign a subjective representation to each taxon based on occurrence. Abundance classes most often are assigned on the basis of a visual estimate of the sample and ordered into abundant, common, and rare categories (Spicer, 1988). Kershaw (1973) noted that this scheme often underestimates small (fragmentary) taxa, and differentiation between the ranks can vary among individual observers. Adjustments of the method that impose a semi-quantitative approach include the modified scale of Braun-Blanquet (1932; Podani, 2006) and the Domin scale, which are based on an estimated percentage of cover for each taxon. These methods appear to be more robust and can be accomplished more rapidly when used in compression-impression macrofossil paleoecological analyses than estimates of cover using point counts (Spicer, 1988).

Autochthonous assemblages, those where stems and derived litter are preserved in situ, permit the most complete data collection. In such instances, it may be possible to evaluate the stature of individual plants, both herbaceous (Wing et al., 1993; Gastaldo et al., 2006) and woody (Williams et al., 2003a), including using mathematical estimates based on general studies of plant growth allometry (e.g., Niklas, 1994). Perhaps most importantly, such assemblages may permit evaluation of the density of individuals within the community, if the exposure allows, using both simple direct measurements and various means of calculating nearest-neighbor patterns (e.g., Wnuk & Pfefferkorn, 1984, 1987; Gastaldo, 1986; DiMichele & DeMaris, 1987; DiMichele & Nelson, 1989; Wing et al., 1993; DiMichele et al., 1996; Cúneo et al., 2003; Williams et al., 2003a, b). Such analyses can provide insight into vegetational structure in addition to the proportional taxonomic composition, which is the currency of most paleoecological analyses. In some cases, such as the Late Cretaceous flora found beneath an ash bed by Wing et al. (1993), it could be shown that species diversity and relative abundance were not correlated; angiosperms, although represented by the most species, did not account for the highest biomass in the assemblage, raising questions about studies that assume a rough equivalence of taxonomic richness and ecological dominance. Biomass contribution from individual taxa also can be calculated in autochthonous assemblages, resulting in productivity estimates that can be compared with Recent communities (e.g., Williams et al., 2003b).

Spatial sampling. Spicer (1988) noted at the time that megafossil-assemblage sampling had been a somewhat haphazard process. He proposed a more codified set of strategies to be applied to bedding surface (two-dimensional) and volumetric (three-dimensional) samples. Several of these methods are effectively the same as neoecological sampling practices. These techniques can be supplemented by others, including bulk sieving of sediment and can provide additional data on the oft-overlooked meso- and micro-fossils (Eklund et al., 2004). The disarticulation of aerial plant parts is notorious for causing overrepresentation of some taxa and underrepresentation of others, relative to their proportional abundance in the parent vegetation (e.g., Burnham et al., 1992; DiMichele & Phillips, 1994). This means that various measures of taxon abundance in the fossil sample may be irrevocably biased, though correctable in some cases. The first step is to recognize that there rarely will be a 1:1 correlation between the quantitative composition of a parent vegetation and that of the derived fossil assemblage, whether autochthonous (Burnham et al., 1992; Burnham, 1997) or parautochthonous (e.g., oxbow lakes; Gastaldo et al., 1989).

For those quantitative studies that use quadrat-based methods, the size of the quadrat selected and the number of quadrats assessed are dependent on many factors, including logistical hurdles. The details of the sampling strategy will be dictated by the size and number of available outcrops, the degree of cementation and mineralization of the matrix, and the time and resources available to carry out the study (particularly if one is working under time constraints in a mine, or on another continent when the best localities are found at the end of a field season), meaning the choice is often less than ideal. In concept, the heterogeneity and scale of plant-part size have to be taken into account when selecting a suitable quadrat size. Spicer (1988) suggests a pilot study during which a pre-survey taxon/area curve (similar to a collection curve, see below) is developed to determine the minimum quadrat size for a specific assemblage. For example, it is most common for 0.5–1.0-m^2 quadrats to be evaluated at the outcrop scale where autochthonous leaf litter is concentrated, whereas assemblages in which large prostrate trees are preserved may be resolved more quickly and effectively by larger areas. Burnham (1989) further

developed guidelines for the choice of sampling strategies (few large samples vs. many small samples) based on the heterogeneity of the source vegetation, which can be approximated by a few simple on-outcrop tests.

Most recent studies of fossil plant assemblages consider explicitly the spatial aspect of the sample. Sample replicates, represented by two or more side-by-side samples, permit intra-site sample variability to be assessed and compared with among-site sample variability (e.g., DiMichele et al., 2007). Although such replicates are not samples from exactly the same site, they are as close as can be expected from fossil-field samples. Among-site comparisons require collections from multiple sampling sites (and replicates thereof) along a transect within a single sedimentary environment (e.g., Wing & DiMichele, 1995; Wilf et al., 1998a; Ellis et al., 2003; Gastaldo et al., 2004a; Willard et al., 2007). Transect studies of leaves preserved in modern sediments (Burnham et al., 1992; Burnham, 1993b) have demonstrated limits to leaf mixing in parautochthonous assemblages and, thus, suggest that most square-meter samples represent relatively local, at most half-hectare source areas, if the parent vegetation was a forest.

Data analysis. It is beyond the scope of this paper to discuss in detail the methods that can be applied to the analysis of various kinds of paleoecological data (see DiMichele & Wing, 1988). Data are often overanalyzed. It is frequently possible to grasp and describe major patterns accurately simply by inspection of the data. The techniques used to analyze patterns in modern vegetation usually can be applied equally well to fossil data. These techniques can be found in any number of general references, such as McCune and Grace (2002), and are widely available in many different computer software packages (including free shareware such as the R package, which includes manuals that offer various insights into the techniques and their peculiarities; R Project for Statistical Computing, 2007).

Most paleoecological analyses are searching for interpretable patterns in numerical data, often with an explicit spatial component. Data generally are stored in a matrix of samples by taxa. Sample similarities can be compared with a wide range of metrics, varying from those based on taxonomic presence-absence (e.g., the Jaccard coefficient), rank-order abundance (e.g., Spearman's rho), or ratio-scale quantitative measures of sample composition (e.g., Euclidean distance). These metrics are the basis for assessing sample similarities, although the choice of metric may be influenced by sample size (Archer & Maples, 1987; Maples & Archer, 1988). The most commonly used exploratory multivariate method for the visualization of similarity among samples is ordination, and the most commonly used ordination methods at the present time are nonmetric multidimensional scaling (NMDS) and detrended correspondence analysis (DCA) (e.g., Miller et al., 2001; Holland, 2006; Willard et al., 2007). Data from other sources, such as the sedimentary environment, can be superimposed on these analyses. Classification methods, such as clustering, are used frequently in conjunction with exploratory methods (e.g., Liang, 2004). Hypothesis testing, regarding the existence of structure in the data, can be carried out with the use of such methods as analysis of similarity (ANOSIM). Methodologically similar to NMDS, ANOSIM examines variation in taxonomic compositional data within and between predefined samples. Discriminant function analysis is a hypothesis-testing classification method that employs a priori–defined groups to develop a classification algorithm that then can be used to assign unknowns to one of the existing groups with a specified degree of confidence.

Recently, several workers (e.g., Wing & Harrington, 2001: fig. 9) have plotted ordination first-axis sample scores against time to detect temporal patterns of change in similarity among samples. This is a powerful visual technique that can quickly reveal vegetational responses to environmental changes in time.

Spatial analyses of in situ vegetation may be reported in two (horizontal space) or three (horizontal space and vertical canopy height) dimensions (e.g., Wnuk & Pfefferkorn, 1987; Wing et al., 1993; Williams et al., 2003b; Gastaldo et al., 2004b). Additionally, the analysis may include various kinds of nearest-neighbor statistics to determine if plant distributions are random, ordered, or clumped (e.g., DiMichele et al., 1996; see Hayek & Buzas, 1997).

A final comment on method involves sample standardization. Under many circumstances, particularly when taxonomic richness is the objective of a study, it will be necessary to standardize sample size in order to reduce the biases of collecting intensity. This is generally accomplished with a method known as rarefaction (Raup, 1975). With this method, a distribution of samples of different sizes is created by repeated resampling from the original quantitative sample. This is done for each sample in the analysis. Then a standard sample size is chosen for the entire sample suite, drawn from the resampling curves created for each of the individual samples (for examples of the use of this method in plant paleoecology, see Wing & DiMichele, 1995. or Wilf et al., 2001).

RECONSTRUCTING THE PAST

Reconstruction of the past, in either visual or descriptive form, of individual species or of entire landscapes, is one of the major ways in which paleontologists make their work accessible, both to specialists and nonspecialists. Throughout the following discussion, a significant concern is the extent to which proportions of species are represented accurately in assemblage reconstructions. Of even greater concern is the degree to which the biologies of the individual plants are captured and integrated into the landscape reconstruction. The implicit dynamics of these past systems are in large part implicit in the portrayal of the reconstructed plants. The main fault of restorations, especially for those that purport to represent the pre-angiosperm world, is the implicit assumption that the plants can be analogized to extant angiosperms with life histories, growth habits, reproductive biologies, and stand characteristics that might be found in a local temperate woodland, so familiar to Northern Hemisphere scientists. This led Pfefferkorn (1995: 389) to label the paleobotanical community, on average, as "temperate climate chauvinists." Take, for example, the ever-present uprooted lepidodendrid trees in Pennsylvanian wetland reconstructions, suggesting blowdown and uprooting as major disturbance elements in such assemblages. In fact, there is virtually no evidence ever reported for uprooting of these trees, or the tree-pit soil disturbances and tree gaps that accompany such events. Equally suspect are the many representations of dense, dark canopies in Carboniferous lepidodendrid forests, given that for many of the species the plants had no crowns until late in life. Such assumptions and analogies to modern vegetation are present to various degrees in reconstructions of the past from any time in geological history. Thus, it may be best to make clear statements about the assumptions regarding the plants and vegetation and, roughly, the confidence that can be placed in a particular reconstruction when offered for general consumption.

Disturbance and succession. The ecological dynamics of past communities, like those of today, are strongly influenced by disturbance and subsequent patterns of succession. Perhaps the most commonly studied disturbance agent is fire. An excellent review of the distribution of fire through time and its relationship to atmospheric oxygen levels is provided by Scott and Glasspool (2006). Wind disturbance, also common in the fossil record, is somewhat more difficult to document. Wnuk and Pfefferkorn (1987) documented a blowndown forest of Pennsylvanian age through access to large bedding surfaces exposed in a coal mine and excellent sedimentological control. Flood disturbance has been documented for a variety of deposits throughout the fossil record (see citations below), the problem being the differentiation of flood disturbance from flood burial. Disturbance in volcanogenic landscapes has been described by Taggart and Cross (1980), Taggart et al. (1982), and Cross and Taggart (1982) in Miocene-age deposits and by Scott and Galtier (1985), as disturbance affected the evolution of early ferns, from the Mississippian of Europe.

Succession following disturbance is more difficult to document. Analyses of vegetational responses to fire need to be done at a finely resolved level, such as the study of Arens (1993), who analyzed a plant succession across a charcoal layer in Pennsylvanian-age deposits by taking small samples across the disturbance horizon. Pryor (1993, 1996) analyzed patterns of succession in a Pennsylvanian-age peat deposit using statistical patterns of plant co-occurrence in coal ball macrofossils. Also studying coal-ball permineralizations, Raymond (1988) analyzed succession during peat accumulation through patterns of successive root penetration. DiMichele and Phillips (1988) correlate patterns of plant response to disturbances within coal beds, again using macrofossils, by linking sedimentological features to recurrent assemblages. Many studies of coal palynology have revealed patterns of vegetational responses both to disturbance and to directional changes in the physical environment of the peat body through time of accumulation, generally related to hydrology and nutrient availability (e.g., Smith, 1962; Grady & Eble, 1990; Willard, 1993; Eble, 1999a, b; Figueiral et al., 1999).

Family portraits: Lots of plants but not much accuracy. Museums are populated by reconstructions of past worlds—dioramas and paintings and combinations thereof. These reconstructions of the past now can be found in abundance and readily on Internet websites, in textbooks, and in various popular venues. They generally are agglomerations of representative species from each of the main plant groups living at a particular time and in a particular region (for example, the wet tropics, a broad dry steppe, or a periglacial area). Such reconstructions often mix together plants from different subhabitats, portray density unrealistically and, overall, can be terribly inaccurate as ecological depictions of the past. Add animals, at excessive densities and in unlikely combinations, and these reconstructions become little more than family portraits, time-averaged gatherings of extinct organisms.

Family portraits have their role, in that few places in the past are sufficiently well known or, if well known, are insufficiently diverse to make for a captivating reconstruction, one that passes along the sense of strangeness of a lost world. In their diversity, family portraits offer the viewer an abstract of the past. Thus, fossil family portraiture has its place and will not be displaced by reconstructions that aim at greater accuracy. Improvements could be made by paying more attention to autecology in particular.

Statistical reconstructions: Reconstructions based on the law of averages. More accurate representations of past vegetation have been based on data from actual paleoecological samples. Because most of such samples are from parautochthonous assemblages (see discussion above), the samples are representations of the original flora, subject to various kinds of taphonomic modifications. Thus, most paleoecological reconstructions based on data are based on some form of likelihood estimate, when the data are considered within the context of the various aspects of taphonomy. Such reconstructions may lack the representational breadth of family portraits, depending on the amount of the landscape they represent.

Statistical reconstructions often are based on the output of numerical analyses, particularly ordinations or cluster analyses of quantitative samples. Such samples provide a sense of recurrent taxonomic associations and may reveal gradients, outliers, and the patterns of relative commonness or rarity of particular combinations of taxa. In some instances, no actual image is produced from such analyses and the reconstruction is descriptive. Broadly considered, statistical reconstructions may also be derived from detailed sedimentological analyses in which plants are related to depositional environments and thence back to distribution on a larger landscape.

Examples of statistically reconstructed landscapes have grown in recent years. Some examples include schematic reconstructions based on various statistical summaries of data (e.g., DiMichele et al., 2002; Liang, 2004; Ricardi-Branco & Rösler, 2004), detailed artistic reconstructions based on quantitative analyses (LePage & Pfefferkorn, 2000; Falcon-Lang et al., 2001; Johnson & Raynolds, 2002; Willard et al., 2007), and descriptive reconstructions based on a variety of quantitative approaches and degrees of depth of statistical analyses of data (e.g., Spicer & Hill, 1979; Phillips & DiMichele, 1981; Raymond & Phillips, 1983; Wing, 1984; DiMichele et al., 1991; Pryor, 1996; Gemmill & Johnson, 1997; Wilf et al., 1998a; Figueiral et al., 1999; Howe & Cantrill, 2001; Johnson & Ellis, 2002; Spicer et al., 2002; Hofmann & Zetter, 2005; Martín-Closas & Galtier, 2005; Wehrmann et al., 2005).

Johnson (1999) discusses in detail the procedures that went into the production of numerous reconstructed landscapes in the Prehistoric Journey exhibit of the Denver Museum of Nature and Science. These reconstructions are based on detailed field sampling of exposures and, thus, represent local views of much broader landscapes, not intended to be family portraits. Examples represented in this exhibit are illustrated in Johnson and Raynolds (2002) and include the Pennsylvanian-age Hamilton Quarry of Kansas (Rothwell & Mapes, 1988), the biota from the Late Cretaceous upper Hell Creek Formation of the northern Great Plains (Johnson et al., 2002), and the exceptionally high-diversity early Paleocene Castle Rock flora of Colorado (Johnson & Ellis, 2002; Ellis et al., 2003).

The Castle Rock flora demonstrates the fine line that separates a parautochthonous assemblage from an autochthonous assemblage. Preserved in a catastrophic flood, the Castle Rock flora is represented by a buried litter horizon with in situ tree trunks with roots in place in a subjacent paleosol. A later litter horizon (possibly derived from dying trees—compare with Burnham and Spicer [1986]) is preserved above the initial flood deposit (Ellis et al., 2003: fig. 4), buried by a second flood event. The abundance patterns of leaf distribution in transect samples and relative positions of disarticulated individual plants demonstrate minimal transport. Thus, this fossil deposit probably preserved regional spatial patterns and is similar to other stands of plants catastrophically buried by floods, where vegetation baffles sediment-laden water, inducing burial with minimal transport (e.g., Andrews et al., 1977; Allen & Gastaldo, 2006).

Direct reconstructions: Raising the dead. Possibly the most accurate reconstructions of past landscapes come from sites that were buried catastrophically by air-fall or waterborne volcanic ash, sediment carried by flood waters, often associated with low areas created by seismic activity, or by the encapsulation of vegetation by precipitation of minerals. The reconstruction of such vegetation is done, essentially, by raising the flattened vegetation backup, hence the label as "direct."

Burnham and Spicer (1986) described vegetation buried by and preserved by volcanic ashfall near El Chichón volcano in Mexico—an initial burial of the forest-floor litter, ground-cover plants, and bases of trees, with a second layer of leaves preserved above the initial bed, representing the leaves from the standing forest, live at the time of the ashfall. A similar deposit was reported by Wing et al. (1993)

from Big Cedar Ridge, Late Cretaceous of Wyoming, buried by air-fall volcanic ash. In situ preservation of the vegetation revealed that ferns were the dominant major group, whereas angiosperms were the most diverse at the species level, demonstrating that diversity within higher taxonomic groups is not necessarily an indicator of dominance of that group, measured as biomass. Other examples of in situ floras buried by ash have been described by Wagner (1989) from the late Pennsylvanian of Spain, and Pfefferkorn and Jun (2007) from the Early Permian of China. in each case preserving plant stems and litter and allowing spatial and whole-plant reconstructions.

Catastrophic burial by flood-borne sediments often is associated with seismically induced changes in elevation, such as those accompanying the formation of Reelfoot Lake, Missouri, and the burial of the forest growing on that site (Penick, 1981). In rapidly subsiding basins proximate to sediment source areas, forests may be buried autochthonously in multiple, closely spaced layers (Gastaldo et al., 2004a), and even in tectonically less active areas, seismic activity may be essential for the creation of accommodation space and the in-place burial of forests (DiMichele et al., 2007). Autochthonous vegetation buried by flood-borne sediment can be found throughout the fossil record of terrestrial plants. Examples include the Devonian Trout Valley flora of Maine (Andrews et al., 1977; Allen & Gastaldo, 2006), numerous cases of Carboniferous lowland, wetland tropical vegetation (Gastaldo, 1992b; DiMichele & DeMaris, 1987; DiMichele & Nelson, 1989; DiMichele et al., 1996; Gastaldo et al., 2004b; Calder et al., 2006), high-latitude forests from the Permian (Taylor et al., 1992), Triassic (Cúneo et al., 2003) and Cretaceous (Falcon-Lang et al., 2001) cycadeoid stands (Wieland, 1916), and in situ fern thickets (Cantrill, 1996) of Cretaceous age, and younger vegetation, such as that of the Castle Rock flora mentioned above (Ellis et al., 2003). In all of these instances, T_0 spatial aspects of the original vegetation are preserved.

The most unusual way to preserve vegetation is by entombment in mineral matter precipitated around the standing plants. The best example of this kind of preservation is the Devonian Rhynie Chert, preserved in travertine deposits from mineral-laden hot springs (Rice et al., 2002). The anatomical preservation of the plants and animals in this deposit is spectacular. In addition, different assemblages of species, plant associations in particular sedimentary environments, and successional patterns have all been identified in what amounts to one of the most completely reconstructed fossil ecosystems.

Autochthonously preserved plant deposits will have their greatest impact when many are known, especially at multiple stratigraphic horizons in close succession, and before and after major extinction boundaries. This is an area in need of much more attention, and in which the gradual accrual of data will eventually provide a richer array of data points for comparison.

PALEOBIOGEOGRAPHY

Paleobiogeography is an increasingly important component of paleoecology. Such studies of plants were among the biological patterns that lent strong support to the idea of continental drift, prior to the widespread acceptance of plate tectonics (see Chaloner [1959] for review), and, later, helped refine study of continental positions (e.g., Ziegler et al., 1996; Berthelin et al., 2003). Vegetational studies also are of importance in the ground-truthing of climatic models (e.g., Wing & Greenwood, 1993; Upchurch et al., 1999; Beerling & Woodward, 2001; Rees et al., 2002; Poulson et al., 2007). The resolution of paleobiogeographic studies is, of course, much better in younger rocks, something paralleled by paleogeographic reconstructions. Furthermore, although basic patterns were apparent in some of the earliest studies, the degree of resolution has improved significantly through time, with improved taxonomies and better paleogeographies.

Paleozoic. The Paleozoic is characterized by a combination of major evolutionary changes in plants, major changes in the relative positions of continental landmasses, high levels of variation in atmospheric composition and global climate, and great variation in sampling intensity at different times and places (DiMichele & Hook, 1992). Consequently, from the Silurian to the end of the Permian there really was a series of worlds in which plant distributional patterns were changing, sometimes dramatically, through time. There also are time intervals of considerable uncertainty due to poor sampling, intercalated among times of rich sample density during which subtle patterns can be detected (e.g., Edwards, 1990; Laveine et al., 2000). Through most of the later Paleozoic, continental landmasses were gradually coalescing into the Pangean supercontinent, resulting in many land-based connections permitting plant dispersal across wide areas. Thus, for the Silurian and Devonian and certainly for the later Carboniferous and Permian, it has been traditional to subdivide the world into large-scale floristic realms. Sampling evidence has consistently pointed to differentiation of three distinct regions: equatorial/low latitude, and north and south temperate high latitude (e.g., Chaloner & Lacey, 1973; Chaloner &

Meyen, 1973; Wnuk, 1996). Ziegler and colleagues (Ziegler et al., 1981; Rowley et al., 1985) added the element of climatic zonation, differentiating floristic provinces. Later, Ziegler (1990) and Rees et al. (1999, 2002) took an explicitly climate-based approach, recognizing ancient biomes as climatically bounded vegetational units, paralleling vegetational distribution on the modern earth (Walter, 1985). Ziegler et al. (2003) argue persuasively that atmospheric circulation patterns are the major controls on vegetational distribution, with due regard for the effects of elevation and paleogeographic locations of moisture sources (e.g., Ziegler et al., 1997).

Summaries of Silurian and Devonian paleobiogeography, the most problematic of the Paleozoic, can be found in Raymond et al. (1985, 2006), Raymond (1987), Edwards (1990), and Raymond and Metz (1995); these analyses suggest some largely latitudinal floristic differentiation with limited taxonomic overlap between regions. The much greater abundance of fossil plant collecting sites from the Carboniferous yields a more detailed understanding of phytogeography at that time. General summaries for the Mississippian include van Der Zwan et al. (1985), Raymond (1985), Raymond et al. (1985), Scott and Galtier (1996), and Raymond (1997). Iannuzzi and Pfefferkorn (2002) recently presented a case for a previously unrecognized warm-temperate floral belt in the late Mississippian of the paleo-Southern Hemisphere, demonstrating the continuing need for field sampling and refinements of the systematic framework on which these floristic analyses rest.

For the Pennsylvanian and Permian periods, there are a relatively large number of analyses of plant distributions, including some on fine spatial scales. The classic papers are those of Chaloner and Lacey (1973) and Chaloner and Meyen (1973), which argue for the progressive differentiation of floristic regions throughout the later Paleozoic, from one to three to five. Thirty years later, Rees et al. (1999, 2002) and Ziegler et al. (2003) had subdivided the late Paleozoic world more finely and demonstrated considerable similarity to patterns in the modern world. Fine-scale refinements of these broader patterns include those of Laveine and colleagues for the Pennsylvanian (Laveine et al., 1989, 1993, 2000; Laveine, 1997). These authors tracked the migration patterns of pteridosperms across the tropics and suggested significant geographic rearrangements of the positions of microcontinental landmasses in the eastern Tethys. Similarly, for the Permian, LePage et al. (2003) have demonstrated yet-to-be-understood physical connections permitting overlap of north temperate Angaran floral elements with those more typical of Euramerican equatorial regions, though principally extrabasinal equatorial elements. Broutin and colleagues (Broutin et al., 1995, 1998; Fluteau et al., 2001; Berthelin et al., 2004; Broutin & Berthelin, 2005) have demonstrated complex mixing of floras along the margin of the tropical regions, bringing together floras from western and eastern portions of the tropical belt with those from more southerly temperate regions. Cuneo (1996) and Iannuzzi and Rösler (2000) have examined phytogeographic patterns in the Southern Hemisphere during the Permian. Pant (1996) summarized paleophytogeographic patterns in India during the late Paleozoic. Li and Wu (1996) examine such patterns in China, and Oshurkova (1996) compares north-temperate Angaran and equatorial Euramerican floristic trends.

Mesozoic. During the Mesozoic, plants recovered from the major ecological disruption at the Permo-Triassic boundary, the Earth's climates were warm, at times from equator to pole, large animals dominated the herbivorous fauna for much of the time, and the angiosperms began to diversify and dominate some ecosystems. Consequently, as with the Paleozoic, many factors impinged on plant distribution and a simple summary of plant paleobiogeography during this time is not really possible.

During the Early Triassic, low latitudes and low-elevational areas of the Earth appear to have been dramatically altered, probably in terms of their biogeochemical cycles, to such a degree that fossil floras are rare. There certainly were extinctions at or near the Permo-Triassic boundary, the extent of which, however, is poorly understood. Despite claims of devastation (e.g., Retallack et al., 1996), it is clear that most major plant clades survived and vegetation was well along in the process of recovering by the Middle Triassic (Looy et al., 1999; Kerp et al., 2006). Ziegler et al. (1993, 2003) summarize some of the physical and biotic changes that occurred during the Triassic.

The Jurassic appears to have been a time of relatively long-term climatic uniformity over much of the globe, following a period of global warming at the Triassic–Jurassic boundary (McElwain et al., 1999). By tying climatically sensitive sediments (especially coals and evaporitic rocks) to the distribution of plants, Rees et al. (2000) concluded that maximum plant productivity during the Jurassic was concentrated in the midlatitudes, where the diversity of plant major groups also was highest. The tropics, in contrast, were dry and supported little if any rainforest vegetation; rather, such areas were populated by xeromorphic conifers and cycadophytes. Polar regions tended to be dominated by deciduous conifers and ginkgophytes. They further conclude that there were five major biomes that remained latitudinally stable

throughout the period, moving across the continental landmasses as the positions of the continents changed. Plant diversity followed the patterns of maximum climatic equability, being highest in the midlatitudes, dropping off toward the equator and poles (Rees et al., 2004); these authors also note a discrepancy between the distribution of plant and dinosaur fossils, which they attribute to taphonomic factors and argue that dinosaurs were most abundant in dry, savannah-like habitats rather than forests. Skog (2001) considered the biogeographic patterns of evolution of leptosporangiate ferns during the Mesozoic and found that most are known from and may have evolved in the midlatitudes or higher, associated with moisture availability.

The Cretaceous has been studied extensively by climate modelers. Some paleobotanical studies that have addressed Cretaceous climate, including plant-climate feedbacks, include those from Spicer et al. (1994, 1996), Upchurch et al. (1998, 1999), DeConto et al. (1999), and Beerling and Woodward (2001). Cretaceous plant productivity and biodiversity were broadly similar to those of the Jurassic in being highest at midlatitudes, where climates were most favorable (Spicer et al., 1993). However, there may have been high levels of terrestrial productivity in some areas of the tropics if continental and atmospheric circulation patterns favored high rainfall, given the climate-biome models of Upchurch et al. (1999). Certainly, peat accumulated at midlatitudes in western North America (Ziegler et al., 1987). Climate changes just prior to the end of the Cretaceous, particularly global warming, have been suggested.

Cenozoic. By the Cenozoic, the amount of geographic and elevational coverage preserved in the fossil record is so much greater than that known or preserved in the Mesozoic or Paleozoic that patterns of plant distribution can be evaluated at a much higher level of detail than in deeper time. For example, Manchester (1999) provides a broad review of North American Tertiary floras, noting a long and well-documented record of floristic exchange among the major Northern Hemisphere continents during this time interval. Similarly, Jacobs et al. (1999) follow the history of grass-dominated ecosystems during the Cenozoic, lending support to a complex history prior to appearance of extensive grasslands in the Miocene. Retallack (2001c) has also reviewed the origin and spread of grass-dominated ecosystems and argues that the long-term co-evolution of grasses and grazers may have contributed significantly to cooling, drying, and climatic instability. The most recent studies of the origin of grasslands have used phytolith evidence (Strömberg, 2002, 2004, 2005) and suggested that the evolutionary radiation of grasses and grazers was decoupled. Prasad et al. (2005), through the examination of grass phytoliths in dinosaur coprolites, were able to determine that grasses were present in significant enough numbers during the Cretaceous to be a food source for dinosaurs. Burnham and Graham (1999) were able to examine the pattern of Neotropical rainforest species composition and turnover from the Miocene to the Recent by a combination of paleobotanical and neobotanical data. They show that plant migrations did not follow those of animals after the formation of the Panamanian isthmus three million years ago; rather, the southern forests have remained relatively free from major, ecologically disruptive invasions. In addition to larger-scale biogeographic studies, it is possible to detect elevational controls on vegetation in Tertiary-period deposits because of the preservation of basins at higher elevations, typically eroded in deeper time (e.g., Taggart et al., 1982; Wehr & Manchester, 1996). The point of these examples is to demonstrate the high degree of spatial and temporal resolution that can be achieved in the study of the biogeography of Cenozoic floras and major plant lineages, to a degree not comparable in deeper time. In contrast to this generality, Collinson (2001) notes that the Cenozoic ferns, although often well known and with distributions exceeding those of their extant descendants, are in some cases more poorly known than ferns from the Mesozoic and Paleozoic, in large part because they are less abundant elements of Cenozoic vegetation than ferns from these earlier times.

Applied Paleoecology

The obvious applications for paleoecological data sets are in the assessment of spatial (within time units at the resolution of age or stratigraphic sequence) and temporal (across time at the resolution of epoch or period) patterns from which (1) communities and ecosystem structure can be evaluated (e.g., Taylor et al., 1992; Wing et al., 1993; Mosbrugger et al., 1994; Falcon-Lang & Cantrill, 2002; Gastaldo et al., 2004b; Falcon-Lang & Bashforth, 2004, 2005; Falcon-Lang, 2006; DiMichele et al., 2007); (2) governing principles of stasis, turnover/replacement, and extinction and assembly laws can be tested (e.g., Knoll et al., 1984; Wolfe & Upchurch, 1986; Pfefferkorn et al., 2000; DiMichele et al., 2001b, 2002, 2004; Willard et al., 2007); and (3) phytogeographic realms can be delimited (e.g., Raymond, 1985; Raymond et al., 1985; Spicer et al., 1993; Rees et al., 2000, 2002; Rees, 2002). Because plant communities are nonmobile, they reflect the unique climatic conditions

governing growth, reproduction, and dispersal across the landscape. Hence, the suite of plant organs, from which the community and/or ecosystem was reconstructed, is a proxy for the paleoclimatic conditions under which the parent plants lived.

LEAVES AND LIVING RELATIVES AS PALEOCLIMATE PROXIES

Nearly a century ago, Bailey and Sinnott (1915, 1916) recognized that leaf morphology could be used as a proxy for climatic conditions, and paleoclimate estimation protocols have long been in the refinement stage. Two principal approaches to climate estimation have arisen (but see also Uhl & Mosbrugger, 1999) based on the morphology of angiosperm fossil-leaf assemblages: leaf margin analysis (LMA; Wolfe, 1979; Wing & Greenwood, 1993; Wilf, 1997) and climate leaf analysis multivariate program (CLAMP; Wolfe, 1993). LMA correlates mean annual temperature (MAT) with the proportion of woody dicot species in a flora having entire (non-toothed) leaf margins. CLAMP takes a multivariate (canonical correlation) approach, involving 31 leaf morphological characters and 11 climate variables (e.g., Spicer et al., 2004, 2005). LMA is faster and simpler to use, but CLAMP offers the computation of a number of different climate variables including MAT, mean cold and warm month temperature, and mean annual precipitation (MAP), and offers the opportunity for additional statistical and graphical manipulations (Green, 2006). For comparison of methods, see recent papers by Jacobs (2002) and Uhl et al. (2007). MAP has been somewhat more difficult to estimate from leaves, and leaf-size analysis has been investigated as a simple and straightforward method to obtain such estimates (Wilf et al., 1998b, 1999).

Proponents for both climate-estimation models continue to test the validity, constraints, and controls on foliar physiognomic responses, often with disparate results when evaluating the same flora (e.g., Wiemann et al., 1998; Gregory-Wodzicki, 2000). With a bias toward preservation within wetlands, Kowalski and Dilcher (2003) found that living dicot communities growing in these North American settings have a higher proportion of toothed leaves that resulted in an underestimation of inferred paleotemperatures ranging between 2.5°C and 10.0°C, regardless of methodological approach. To redress the underestimate, they introduced wet-site regression equations and proposed that predictive equations be redeveloped using sites more analogous with those identified in deep time. Depending on the biome under consideration, both overestimates (e.g., Jacobs, 2002) and underestimates (e.g., Greenwood, 2005) of climate parameters have been reported, with underestimation the result of low-diversity angiosperm floras. But, when high species diversity exists, such as in the paratropics and tropics, there may be a minimum number of taxa above which credible MAT estimates and somewhat less credible MAP estimates can be made. Burnham et al. (2005) found a correlation between species richness and the accuracy of climate estimates in the Neotropics; estimates came within ± 2°C of recorded temperatures when 50% of the highest rank-ordered taxa were used. More accurate estimates were achieved as the taxonomic number increased. When less than 25 taxa were used in an analysis, estimates were outside the accepted ± 2°C margin of error (Wilf, 1997). In their data, a bias toward serrated leaves existed in the data set (comprised of the top 10 rank-order taxa), with this bias disappearing once the top 20 taxa were taken into consideration. Hence, Burnham et al. (2005) concluded that habitat may not be relevant to the bias associated with MAT estimates. Additionally, they found that MAP estimates for all their sites were too low, even when all taxa were used.

The other approach to climate reconstruction is the nearest living relative (NLR; Mosbrugger, 1999) model, where the climatic requirements of the NLR of a fossil taxon is, or assemblage components are, used to estimate the paleoclimate under which the organism(s) lived. Mosbrugger and Utescher (1997) introduced a variation, the coexistence approach (CoA), in which the climate requirements of a fossil assemblage are estimated by using a compilation of the ranges of climatic requirements of each taxon's systematically NLR. Climatic variables (MAT, MAP, etc.) under which all taxa within the assemblage could have grown together are used to develop the estimates. Theoretically, this method can be applied to assemblages where at least one NLR can be identified, but, obviously, resolution increases with an increase in the number of taxa in an assemblage with NLRs. Mosbrugger and Utescher (1997) demonstrate a strong congruence between climate parameters in both Recent and Neogene floras (88%–100% of coexisting taxa). Tests of the CoA against both LMA (Uhl et al., 2003) and CLAMP (Utescher et al., 2000; Liang et al., 2003) demonstrate its applicability to Tertiary assemblages. When compared with LMA, both approaches can produce sensible and consistent results, depending on the flora (e.g., Liang et al., 2003), when the standard error of the leaf-physiognomy paleoclimate data is taken into account. But Uhl et al. (2003) caution that reconstructions based on leaf physiognomy are influenced by factors not related to climate, such as sample size and taphonomy. When results are compared between CLAMP and CoA analyses, lower paleoclimate estimates were calculated using CLAMP for European Mio-Pliocene

assemblages (Mosbrugger & Utescher, 1997; Utescher et al., 2000) and for a Miocene assemblage preserved in diatomaceous lake shales in Asia (Liang et al., 2003). A similar CLAMP underestimate is reported for the Shanwang site when compared with LMA (Sun et al., 2002b).

This begs the question as to whether or not such protocols can be applied to the non-angiosperm world. Most non-angiosperm deciduous taxa have no NLRs. Thus, to date, climate estimates based on such taxa have met with only partial success. In the deepest time analysis to attempt LMA, Glasspool et al. (2004) evaluated Permian-age Cathaysian gigantopterids, which have angiosperm-like broad leaves; the results of the analysis are limited, however, by lack of another independent proxy for temperature. Another taxon, *Ginkgo* L., is interpreted to have had similar ecological distribution since the Mesozoic in disturbed stream-side and levee environments (Royer et al., 2003). The ecological constraints of Late Paleozoic representatives (*Dicranophyllum* Grand'Eury) are still uncertain; the taxon has been found associated with Lower Permian Southern Hemisphere coals (Guerra-Sommer & Cazzulo-Klepzig, 2000). And, because living *Ginkgo* is monospecific (*G. biloba* L.) and there are no unequivocal natural stands today (unlike the recently discovered Wollemi Pine, Araucariaceae; Chambers et al., 1998), there is a high probability that Ginkgoaleans were adapted widely to a variety of habitats (see Rees et al., 2002). On the other hand, stomatal trends of fossil *Ginkgo* are believed to parallel those of the living taxon grown under varying atmospheric gas concentrations (Beerling & Royer, 2002a; see below).

Due to the seeming obstacle of ecological homology, other workers have utilized permineralized wood assemblages to estimate paleoclimatic parameters (e.g., Chaloner & Creber, 1990). Recently, though, Falcon-Lang (2005a) and Poole and van Bergen (2006) have demonstrated that the relationship between climate and growth-ring parameters in modern trees, in part, invalidates the use of fossil woods as climate proxies. This is due to unconstrained variability in tree response to climate-forcing (i.e., growing-season length and growth conditions, seasonality, productivity) on a global scale, applicable to both angiospermous and coniferous woods (Falcon-Lang, 2005a, b).

Proxy approaches to understanding ancient ecosystems and the environmental conditions of their formation at whole-system levels have been applied extensively to such settings as peat-forming environments, where physical controls and taphonomy often are considered to be conservative and independent of plant composition. There is an extensive literature on peat-to-coal models, including environmental inferences. Models, such as those of Diessel (1986) and coworkers, that would identify modern vegetational equivalents, such as reed-dominated versus woody forest–dominated systems, in Carboniferous forests based on coal macerals do not take into consideration the significant differences in the plants from different times in geological history and, thus, risk inappropriate ecological conclusions. As pointed out by Collinson and Scott (1987) in a comparison of Carboniferous lycopsid-dominated systems with Cretaceous to Recent taxodiaceous-dominated systems and DiMichele and Phillips (1994) in an examination of Carboniferous systems, the qualities of the plants cannot be ignored.

STOMATAL DENSITIES AS CLIMATE INDICATORS

The developmental response of leaves to elevated pCO_2 as reflected in leaf stomatal density has garnered great attention over the past decade (e.g., McElwain & Chaloner, 1996). Application and limitations of the approach have been reviewed elsewhere (e.g., McElwain, 1998; Royer, 2001; Beerling & Royer, 2002a; Roth-Nebelsick, 2005), and the reader is directed to these contributions. An inverse relationship exists between the pCO_2 and stomatal index (SI; Poole & Kürschner, 1999) such that stomatal indices (the ratio of stomata to epidermal cells) decrease as the concentration of atmospheric CO_2 increases. This inverse response is found in both angiosperms and gymnosperms (e.g., Kouwenberg et al., 2003) but is not limitless. It has been demonstrated in modern angiosperms that not only will different species within the same genus reach their own SI limit at different pCO_2 (e.g., *Quercus* L.; Kürschner, 1997), but species of the same genus (*Quercus*) grown under identical climate and atmospheric gas conditions exhibit statistically significant differences in both stomatal density and SI values (Cantor et al., 2006). Hence, caution is necessary when using this approach in deep time (Kürschner, 1997).

There have been attempts to apply non-angiosperm stomatal indices in other parts of the pre-angiosperm record. For example, a number of researchers have used the Ginkgoales to examine geological trends in pCO_2. Retallack (2002) used extant *Ginkgo biloba*, in part, to calibrate responses of fossil *Ginkgo* species back into the Late Triassic, and extended this even further back into the Permian, using pteridosperm taxa with purported evolutionary affinities to the Ginkgoales. The assumption, of course, is that all these related species exhibited the same physiological and developmental responses (Beerling et al., 1998) at

all times and places. Chen et al. (2001) examined modern *G. biloba* and found significant differences in stomatal patterns relative to timing of leaf maturation, leaf development, placement on short versus long shoots, canopy position, and sexuality. They concluded that only mature leaves of *G. biloba* should be used in any analysis. They (Chen et al., 2001) then matched stomatal estimates of pCO_2 from four extinct species of *Ginkgo*, ranging in age from Early Jurassic to Early Cretaceous, to the pCO_2 curve of Berner (1998) and found congruence for only two. NLRs for Jurassic cycadaleans also have been used (e.g., McElwain et al., 1999). Using stable carbon-isotope data, Beerling (2002) extended the approach to Carboniferous arborescent lycopsids using extant tropical *Lycopodium cernuum* L. for calibration purposes, although acknowledging closer affinity to extant *Isoetes* L. Values derived from the stomatal indices of Carboniferous arborescent lycopsids were consistent with the independent indicators of a drop in atmospheric pCO_2 concentration during the Late Paleozoic glaciation.

In the absence of one or more NLRs, McElwain and Chaloner (1995) chose to use nearest living equivalents (NLEs), defined by them as Recent taxa that grow in a comparable ecological setting and/or are structurally similar, in their evaluation of Devonian (*Juncus* L. = *Sawdonia* Hueber and *Aglaophyton* D. S. Edwards; *Psilotum* Sw. = *Aglaophyton*) and Carboniferous (*Araucaria* Juss. = *Swillingtonia* A. C. Scott & Chaloner and *Lebachia* Florin) plants. They found a marked contrast in all stomatal parameters between the extant and the extinct taxa. The Devonian fossil taxa exhibited significantly higher SI values than their NLEs, whereas values for the Carboniferous taxa plotted within the range of extant araucarians. These results were consistent with Berner's (1991) GEOCARB model, confirming a massive atmospheric drop in CO_2 from the Early Devonian through the Permo-Carboniferous. Again, using the NLE approach, with ginkgoaleans and cycadaleans, McElwain et al. (1999) linked major species turnover at the Triassic–Jurassic boundary to a 3°C–4°C increase in global temperature.

For deep-time taxa without either an NLR or NLE, physiological and developmental responses have been inferred using even more generalized assumptions about stomatal responses. For example, Hesselbo et al. (2003) used the stomatal densities (SD) instead of SI values in plants from a Middle Jurassic sequence of Yorkshire to develop a general pattern. Although SI is more reliable than SD (Poole & Kurschner, 1999; Royer, 2001; Beerling & Royer, 2002b), preservational problems dictated use of the latter. Stomata patterns in pre-Mesozoic gymnosperms also have been used to develop proxies for pCO_2. Cleal et al. (1999) evaluated stomatal parameters in the pteridosperm *Neuropteris ovata* Hoffmann across the Moscovian–Kasimovian (Wespthalian–Stephanian) boundary. Their results suggest an increase in atmospheric CO_2 in the very early Kasimovian, consistent with changes in vegetational composition and carbon sequestration (DiMichele & Phillips, 1996; Cleal & Thomas, 2005). More recently, Wang and Chen (2001) extended the application to the extinct arborescent lycopsids, using cuticles recovered from leaf cushions and distal sporophyll laminae. The shift in values across the Upper Permian of China, tied to a shift in lithofacies, is attributed to a change in the physiological response of these taxa from C_3 to CAM photosynthetic pathways in response to an increasingly dry climate.

INFERRING PHYSIOLOGICAL RESPONSES OF EXTINCT PLANTS AND THEIR IMPLICATIONS

Paleobotanists are increasingly finding ways to estimate plant physiological functions and responses to environmental conditions. Konrad et al. (2006) developed a model to evaluate photosynthetic capability as it relates to diffusion resistance of the epidermal cells based on the size and depth of stomatal pores and mesophyll parameters (e.g., intercellular air space, mesophyll-cell area, and membrane resistances). They also added functions to evaluate the relationship between assimilation and temperature. The model permits use of stomatal morphologies and densities to calculate past CO_2. Similarly, Royer and Wilf (2006) have proposed and evaluated a gas-exchange hypothesis that explains the relationship between leaf-margin morphology and climate variables. They tested the efficacy of the model, found that it accurately predicted the response of leaves from colder climates, and found an increased efficiency in transpiration and photosynthate production early in the growing season in taxa with toothed margins versus those with entire margins (this also may explain the higher proportion of toothed-leafed taxa in stressed wetlands). Additionally, plant response is enhanced at higher-temperate latitudes (38.8°N, colder temperate Pennsylvania; 35.7°N, warmer temperate North Carolina) where the plants maximize carbon gain when temperature is limiting but moisture and nutrient availability are not. They have concluded that the increased physiological functioning associated with serrated leaf margins may provide a proportionally increasing selective advantage with decreasing temperature (or water stress). This is reflected in empirical correlations used for paleo-temperature estimation. Another recent

hypothesis relating toothed-leaf margins to temperate climates is that of Feild et al. (2005), who suggest that teeth act as points of guttation, a process of relieving flooding of leaf tissues by root pressure on the vascular system. These authors propose that by relieving the negative effects of flooding, guttation permits root pressure to drive leaf developmental expansion. Cool, high-humidity spring weather, combined with moist soils in many temperate regions, would be conditions in which guttation was favored. In addition, this model explains the overabundance of toothed-margin leaves in wet habitats (Burnham et al., 2001; Kowalski & Dilcher, 2003). In contrast to these organ-scale studies, Beerling and Osborne (2002), working at the level of the biome, considered such responses as carbon balance in high-latitude coniferous forests to elevated carbon dioxide levels.

Studies of the leaf economics spectrum in extant plants (Wright et al., 2004) have revealed nonlinear correlations among leaf morphological and physiological characteristics arrayed along a single rate spectrum. At the extremes, what Wright et al. (2004) call quick-return leaves are characterized by high nutrient concentration, high photosynthetic and respiratory rates, short life spans, and low biomass per unit area; at the slow-return end are leaves with low nutrient concentrations, long life spans, low photosynthetic and respiratory rates, and high investment in biomass per unit area (usually in antiherbivore tissues). Correlations of these traits with climate were present, especially when constrained by biome or growth-form/functional group. Wilf et al. (2001) used these morphological traits to estimate leaf life spans and position within the economics spectrum for late Paleocene and Early to Middle Eocene dicotyledonous angiosperm leaves, and found close correlations among herbivory and inferred plant defenses in response to independently assessed changes in paleoclimate. Royer et al. (2005) used and extended these morphological-physiological correlations. Based on a preliminary study (Huff et al., 2003), Royer et al. (2005) quantified univariate correlations among leaf traits and demonstrated statistically significant patterns (number of teeth, perimeter ratio, shape factor, and margin percentage); this latter study also demonstrated several relationships between leaf physiognomy and leaf economics. From these results, Royer et al. (2005) developed a set of refined leaf-climate models using multiple linear regressions.

The NLR approach to study of extinct plant physiology has been applied to various plant groups from different times in Earth's history. Perhaps the most explicit comparisons have been made by Feild and colleagues, who have combined studies of angiosperm phylogeny with physiology to examine likely physiological traits of evolutionarily basal angiosperms (Feild et al., 2000, 2001, 2003a, b). In the deeper past, Phillips and DiMichele (1992) considered the consequences for arborescent isoetalean lycopsids of CAM metabolic pathways, which have been found in modern isoetaleans (Keeley & Busch, 1984) and also inferred from carbon isotopic analysis (Raven & Spicer, 1996). Boyce and colleagues (Boyce & Knoll, 2002; Zwieniecki et al., 2004; Boyce, 2005) examined the physiological attributes of leaves and their xylary support systems in modern seed plants and pteridophytes as indicators of possible constraints on the evolution of leaves in primitive gymnosperms and ferns. As part of this, Boyce et al. (2004) examined the physiology of tracheid lignification in a phylogenetically diverse sample of extant vascular plants. They postulated trade-offs between hydraulic transport and support as the evolutionary driving force of lignification patterns. Lignified tracheidal cells are found in plants with small leaves or leaves with needle-like construction. In those plants with larger laminate leaves (ferns and angiosperms), lignification of tracheidal cell walls is decreased, enhancing water transport, with support functions shifted to heavily lignified fiber cells for support.

GROUND-TRUTHING CLIMATE MODELS

Inferences about climate based on plant physiognomy and ecological tolerances casts plants as natural, independent sources of information with which to ground-truth global climate models at continental, hemispherical, and global scales. Such an approach was used by Wing (1991) in a critique of computer models (Sloan & Barron, 1990). The presence of large nonburrowing tortoises, palms, tree ferns, and dicot trees lacking strong seasonal growth rings in Late Cretaceous and Paleogene deposits of western interior North America was considered incompatible physiologically with sustained below-freezing winter temperatures and a high degree of seasonal temperature fluctuations predicted by the models. Wing (1991) extended the biological argument to the entire Tertiary prior to ca. 34 Ma. He found paleontological evidence to be compatible with the climate-simulation parameters in only one instance for the Paleocene and later Eocene. More recently, Fricke and Wing (2004) have evaluated both LMA and stable isotopes, from phosphate in mammalian tooth enamel and freshwater fish scales, to estimate the MAT in the Early Eocene across a North American latitudinal gradient from Ellesmere Island to Texas. They acknowledge that even in areas where both megafloral and megafaunal assemblages occur in

the same stratigraphic section, these are rarely preserved within the same bed (same chronostratigraphic horizon; this also applies to the entire Phanerozoic record). Furthermore, it is difficult to make direct statistical comparisons in MAT between different paleogeographic areas due to age uncertainties because MAT can change significantly over short periods of time (Milankovic time scales). Contrary to the straight-line application of Recent correlatives between these parameters, Fricke and Wing (2005) demonstrate that the oxygen isotope–MAT correlation during the early Eocene was significantly different from the climate proxies obtained through LMA ($\delta^{18}O$ estimates were 0.2°C to 2.1°C higher). They also found that the absolute values for $\delta^{18}O$ differed between the Bighorn, Green River, and Powder River basins of Wyoming, implying that different hydrological regimes existed in these areas. Regardless, this combined approach toward paleotemperature estimates captured a global-warming event at the Paleocene–Eocene boundary not determinable from paleobotanical data alone.

Deeper time climate models are more problematic simply because both abiotic and biotic sources of data are more remote, meaning sparser data, limited modern proxies with which to compare, poorer regional stratigraphic-temporal correlations, and, in some cases, exacerbation of a basic lack of understanding of the complexities of the Earth-climate system. Nonetheless, it is possible to combine geological data and paleontological data in the search for common patterns, allowing climatic inferences based on physical data to be compared to biological changes (e.g., Montañez et al., 2007; Poulsen et al., 2007). Late Paleozoic changes from a cool to warm Earth (Gastaldo et al., 1996) serve as an example of this kind of study. During the Permian, Southern Hemisphere deglaciation occurred in a stepwise pattern, inferred from stable isotopes of marine (Veizer et al., 1999) and paleosol (Royer et al., 2004) carbonates of Euramerica. Three glacial and interglacial pulses have been identified, each distinguished by a $\delta^{18}O$ excursion, with glacial ice present during the late Gzhelian–Asselian (Carboniferous–Permian), across the Sakmarian–Artinskian boundary, and during the Kungurian–Roadian. Complete Gondwanan deglaciation occurred during the Middle Permian (Roadian), although there are some suggestions of later minor ice pulses (Fielding et al., 2005). Preliminary estimates of atmospheric carbon dioxide concentrations suggest that Permian pCO_2 was up to 10 times greater than during the Late Carboniferous, in which a pCO_2 concentration of ~350 ppm parallels the present atmosphere (Montañez et al., 2007). A complex spatio-temporal vegetational response to these cycles and overall increasing pCO_2 is recorded in tropical paleolatitudes of the southwestern U.S.A. This pattern shows a trend from wetland megafloras to those that grew under more seasonal rainfall patterns, many elements of which are typically thought characteristic of the Late Permian and Mesozoic (DiMichele et al., 2001a). The overall megafloral compositional changes are similar in western North America and Central Europe, although correlation problems prevent exact comparisons of timing (Scott, 1980; Kerp & Fichter, 1985; Broutin et al., 1990; DiMichele & Aronson, 1992). Overall, this combined geological-paleontological approach demonstrates a close correlation of changes in the abundances of major groups of plants and inferred changes in atmospheric CO_2, soil moisture, and regional temperature. Major changes in landscape patterns occur at each inflection point of the pCO_2 record rather than at stage boundaries (Montañez et al., 2007), as suggested by global diversity compilations (e.g., Rees et al., 2002). Continental-scale patterns provide insight into the combined effects of changing global climate and paleogeography. However, finer sequence-scale analysis within the regional sedimentological, chronostratigraphic, and geochemical framework captures the nuances of terrestrial ecological response to perturbation.

BIODIVERSITY PATTERNS IN SPACE AND TIME

Applications of synecological analyses have primarily been focused on diversity studies at the continental, hemispherical, and global scales. In general, the systematic diversity of collection sites across an area of interest has been compiled into databases (e.g., The Paleobiology Database, 2007), with all floras binned within a stratigraphic interval (usually stage-level). Spatial and temporal changes can then be evaluated to identify patterns of diversification, range extensions (or contractions), and ecological assembly.

The best resolution of paleobiogeographic patterns is in the extensive Tertiary plant-fossil record, in which there are many extant angiosperm families. The quality of this record has led to many recent studies of group origination patterns, diversification, and range changes relative to present biogeographic distributions (e.g., Tiffney & Manchester, 2001). It is beyond the scope of this review to document all that has been published relative to angiosperm paleobotany in these respects. A few illustrative examples will suffice to demonstrate major research approaches.

Recently, Corbett and Manchester (2004) evaluated the stratigraphic and paleogeographic distribution of *Ailanthus* Desf., a Northern Hemisphere genus commonly encountered as fossils in lake sediments

associated with temperate evergreen taxa. The oldest megafloral remains are known from the Early Eocene of North America and Asia, with the genus appearing in Europe by the Middle Eocene and ultimately achieving a circumboreal distribution. Presently, *Ailanthus* is restricted to the Indian subcontinent, Southeast Asia. the Indonesian archipelago, and northern Australia (violating Wallace's line). Corbett and Manchester (2004) conclude that *Ailanthus* either originated in the Northern Hemisphere and subsequently extended its range southward, or vice versa. Macrofloral evidence exists for the former but is suspect for the latter (there is a dearth of fossil evidence in these geographies). Any attempt to resolve this dilemma would require the use of a molecular phylogenetic analytical approach that may provide evidence for the ecological limits of the ancestral lineage.

Such an approach has been taken by Xiang et al. (2005, 2006) for *Cornus* L. They combined DNA data with morphological traits of extant and extinct taxa. From an extensive set of analyses (PAUP [maximum likelihood], parsimony, and Bayesian phylogeny inference and divergence-time analyses), they conclude that *Cornus* underwent five sequential intercontinental dispersals since origination in Europe. During the late Paleocene, its range expanded into North America via the North American land bridge, with separation and climate forcing isolation (*C. sessilis* Torr. ex Durrand, western U.S.A.) and extinction (eastern and central North America). Range extension occurred into Africa at about the same time, with these taxa retaining an evergreen habit in tropical montane-evergreen forests of eastern Africa. Europe-to-Asia transferral occurred twice, in the mid-Oligocene and mid-Miocene, arriving on this continent also as an evergreen lineage. Documentation of such phylogeographical histories and assemblages becomes the basis for understanding ecosystems and their responses to perturbation (e.g., Collinson & Hooker, 2003).

Extrapolation into deeper time where there are stratigraphic, paleogeographic, and paleoclimatic complications results in much coarser temporal and biogeographic resolution. Rees et al. (2004), for example, used climatically sensitive sediments, plant diversity, and dinosaurs to infer broad geographic patterns for the Late Jurassic (Kimmeridgian and Tithonian, 155.7–145.5 Ma). Using the data compiled by Rees et al. (2000) and plotted within latitudinal bins of 10°, they note low plant diversities in low latitudes where dinosaur remains are well preserved (ascribed to a taphonomic bias and a small number of localities), increasing diversity toward the midlatitudes, and a pole-ward diversity decrease (where megafaunal elements are virtually unknown). High-midlatitude diversity forests were dominated by a mixture of conifers, cycadophytes, pteridosperms, ferns, and sphenophytes. This stands in marked contrast with low-latitude xeromorphic vegetation, characterized by microphyllous conifers and cycadophytes, and polar assemblages dominated by macrophyllous conifers and deciduous ginkgophytes. Although it was only possible to delimit coarse spatial (continental and latitudinal) and temporal (Kimmeridgian, 4.9 Ma; Tithonian, 5.3 Ma) resolution of these vegetational assemblages, they conclude that "an immense biomass and diversity of dinosaurs was apparently supported by sparse vegetation" (Rees et al., 2004: 649) occupying a tropical savannah biome (Rees et al., 2000).

A similar stage-based approach was taken by Rees et al. (2002) for the transition from icehouse to greenhouse conditions in the Permian (Gastaldo et al., 1996), taking into account the northward movement of continents over this interval. Plant-assemblage diversities were compiled at the generic level using taxonomic lists taken from the literature for the Sakmarian (285–280 Ma) and Wordian (267–264 Ma; Rees et al., 1999). These were used in conjunction with dry and wet climatically sensitive lithologies to interpret polar-to-equatorial climate gradients within each stage. To limit spatial and temporal averaging, megafloral data were restricted to inter-locality distances of ca. 100 km and vertically restricted throughout the stratigraphies. The resultant Sakmarian data set consisted of 112 genera from 128 localities (799 occurrences); the Wordian was comprised of 104 genera from 147 localities (1001 occurrences). From these data, nine biomes (tropical ever-wet to tundra) were identified, and the morphological adaptions of the plants within each biome were used to infer precipitation and temperature patterns within the established paleogeographical context. Biomes were compared against computer climate-generated biome models run at two levels of pCO_2 (4×, 8×) to assess the credibility of the interpreted distributions. Rees et al. (2002) found that biome simulations run at elevated pCO_2 matched the empirical data in the equatorial, midlatitudes, and northern high latitudes, although no simulation reproduced the temperate climates in high southern latitudes where alpha diversity is limited to fewer than 15 taxa (Rees, 2002). As Rees (2002) notes, each regional pattern is a reflection of sampling and the effects of regional-level geographic and climatic changes (wet to more seasonal) on different taxa growing in different biomes. In addition, perturbations, extinctions, and turnover at the substage level play a significant role in the perception of broad regional patterns when these assemblages are amalgamated.

Phylogenetic Analyses in Paleoecology

Accounting for the effects of history on ecological patterns and dynamics is rapidly growing in importance. Attempts have been made both to accommodate and to remove the effects of historical patterns in ecology, by considering both phylogenetic relatedness and biogeographic history. The integration of phylogenetic pattern in the analysis of ecological pattern and process leads inexorably toward further consideration of the fossil record. Fossils not only provide direct evidence of the combinations and distributions of extinct organisms, but also permit dating of nodes in cladograms (Crepet et al., 2004), which may prove important in determining when, where, and under what environmental conditions clade ecological traits may have been established. Conversely, if clade membership strongly affects local ecological structure, it may be difficult to examine patterns of convergence among ecosystems from different times and places, and thus, clade membership attempts to account for phylogeny by recasting ecological descriptions in terms of functional groups or ecomorphs.

Including Phylogeny

In neoecology, the consideration of phylogeny has led to findings that contradict traditional views of plant ecological variation. One need not look far to find the opinion that all plants do the same thing, and, hence, much convergence in ecological patterns is to be expected across widely unrelated lineages, rendering history unimportant. However, work such as that of Ackerly (1999), Webb (2000), or Prinzing et al. (2001), for example, or in a number of papers (e.g., Cavender-Bares et al., 2006; Kembel & Hubbell, 2006; Silvertown et al., 2006) in a special issue of the journal *Ecology* devoted specifically to this subject (Webb et al., 2006), suggests that ecological distributions of species or higher taxa tend to be more clustered with regard to spatial and habitat conditions than expected by chance. Thus, all plants are not doing the same thing and there may be strong phylogenetic and other historical effects on biodiversity and the ecological mediation of evolutionary patterns. Such integration of explicit historical considerations and ecological patterns and processes appears to be an area with tremendous potential to have wide, profound effects on many aspects of ecology (see summaries and comments by Webb et al. [2002, 2006], Ricklefs [2006], and Westoby [2006]).

There are some paleoecological studies that have examined or considered ecological patterns in light of phylogenetic patterns. Examples at a relatively finely resolved taxonomic scale include Burnham's study of early Cenozoic elms (1983, 1986), in which the major genera of the subfamily Ulmoideae were characterized morphometrically and with respect to their climatic, particularly temperature, tolerance ranges. In each of three genera, species-level diversifications were found to occur during times of climatic instability in the late Eocene. However, new species had the same basic climatic tolerances as both the ancestral forms and those found in the living representatives today, suggesting strong phylogenetic niche conservatism at the clade level. In a study of another Cenozoic member of the Ulmaceae in the subfamily Celtoideae, *Cedrelospermum* Saporta, Manchester (1989) found that the several known species of this genus were typically colonizers of areas disturbed by volcanic ash deposition; some extant members of the Celtoideae share this peculiar ecology. Although the edaphic distribution of *Cedrelospermum* was narrow, Manchester notes that it was widely distributed through a variety of climatic conditions. Wing and Hickey (1984) examined the systematics and paleoecology of phylogenetic relationships within the Juglandaceae, focusing on the genus *Platycarya* Siebold & Zucc. They found Tertiary *Platycarya* species to be thicket-forming shrubs characteristic of open, early successional habitats. This particular ecology is characteristic of species of this genus up to the present day.

The use of nearest-living relatives to infer the climatic tolerances of fossils, discussed above (Mosbrugger & Utescher, 1997; Mosbrugger, 1999), makes the implicit assumption that clades are conservative in their ecological tolerance ranges. By looking across a flora instead of using a single species, the coexistence approach to estimate such climatic tolerances of extinct forms (Mosbrugger & Utescher, 1997) adds considerable support to the general assumption of clade conservatism in niche breadth.

From a still broader, floristic perspective, Wing (1988a) examined the origin of Eocene and Oligocene floras in the Rocky Mountains and concluded that derived microthermal lineages, those typical of cooler, more climatically seasonal climates, originated in high-elevation areas, whereas the lower elevations were dominated by ancestral megathermal (warm equable climate) forms. Once climatic cooling spread throughout western North America, these lineages migrated into and dominated cooler climates that had become widespread through the lowland regions.

Similarly, DiMichele et al. (2001a) found Early Permian floras dominated by phylogenetically derived conifers and cycads, plants previously known only from Late Permian or Mesozoic deposits, associated with a tongue of sediment in the western Pangean equatorial belt deposited during cooler, strongly

seasonal climates, possibly coincident with a last-gasp pulse of high-latitude glaciation (Montañez et al., 2007). Ancestral forms typically grew in more equable seasonal settings.

Both these examples lend support to models that link evolutionary innovation to the crossing of ecological thresholds (e.g., Wiens & Donoghue, 2004; Rickleffs, 2006) and the establishment of basic clade-level ecological traits during these initial radiations.

At a more global spatial scale, the role of history in constraining hypotheses of evolutionary inferences was considered by Manchester and Tiffney (2001) who, again using elms, demonstrated that inclusion of extinct taxa and information on past geographic distributions not only provided more complete phylogenies and added constraining age data, but permitted significant refinement of interpretations on place and conditions of clade origin and evaluation of age and area hypotheses. They conclude that the fullest picture comes from a combination of neobotanical and paleobotanical data within a phylogenetic context.

At the deepest time level, DiMichele and Bateman (1996), Bateman et al. (1998), and DiMichele et al. (2001b) examined the origin of vascular-plant body plans, roughly equivalent to Linnean classes, during the Middle to Late Devonian and concluded that there was strong overlap between phylogenetic affinity and ecological distribution, with lycopsids occupying primarily wetland habitats, seed plants in terra firma habitats, sphenopsids in a narrow range of aggradational settings, and ferns as opportunistic weeds in terra firma environments. Hotton et al. (2002) found evidence of this same basic clade-by-environment subdivision as far back as the Early Devonian, wherein the ancestral clades (zosterophylls and trimerophytes) of the main Late Devonian (and beyond) body-plan groups were already established along the wet-dry-substrate spectrum.

Perhaps the largest scale and most general study to attempt to understand patterns of ecological convergence and divergence is that of Niklas (2006), who compared allometric patterns of biomass partitioning in aquatic macrophytes, bryophytes, and vascular plants, explicitly examining the patterns within a phylogenetic framework. He found a similar pattern of resource allocation to functionally equivalent body parts that are otherwise developmentally distinct and non-homologous in these different groups. From this, he concluded that there may be a single scaling relationship describing biomass partitioning into stem, leaf, and root for all eukaryotic photoautotrophs. This finding, as Niklas (2006) noted, permits the use of simpler, more general model systems to develop analytical solutions to explain biomass partitioning patterns in higher plants. At this level, the inclusion of explicit phylogenetic consideration leads to the conclusion that at some level of analysis, such considerations can be ignored (see next section).

Similar to studies of allometry within a phylogenetic framework are recent studies of physiology linking fossil and modern plants by Boyce and colleagues (Boyce et al., 2003, 2004; Boyce, 2005), discussed above. This work has permitted convergent patterns in morphology to be identified and linked to underlying physiological controls, revealing certain patterns of trade-off in the basic plant functions, support versus hydraulics. These studies link closely with those of the leaf economics spectrum (Royer et al., 2005, also discussed above), in that plants with small, structurally complex and highly defended, long-lived leaves differ in terms of the lignification patterns in their hydraulic and support systems (having the more primitive condition) from plants with shorter-lived, more physiologically active foliage.

EXCLUDING PHYLOGENY

Just as there have been attempts to include phylogeny and examine the effects of relatedness on ecological patterns, there have been (much more long-standing) attempts to remove such effects. In neoecology, these attempts fall broadly under the study of guilds (Root, 1967; Simberloff & Dayan, 1991) or functional types (Smith et al., 1997). Such approaches implicitly assume that evolutionary relatedness will bias the distribution of plant taxa in space and by resource use pattern, thus impeding comparisons among ecosystems that might otherwise be found to converge (or not) in structure. This approach also aims to make systems comparable in such cases where true morphological convergence exists, but is masked by the use of taxa as the variables by which ecological assemblages are categorized. Functional-type analyses may reclassify plants via multivariate analysis based on their morphological traits, or place them into groups or guilds based on inferred functional roles within ecosystems (Gitay & Noble, 1997; Westoby & Leishman, 1997). This approach may be applied to entire ecosystems as well as to the invididual taxonomic components of those systems (Shugart, 1997).

There have been a few paleobotanical attempts to remove taxon effects, an approach that has been used somewhat more extensively in studies of vertebrate paleontology (see Damuth et al., 1992). Wing (1988b; Wing et al., 1992) placed 52 species of Eocene-age flowering plants, gymnosperms, and lower vascular plants into 12 ecological categories based on foliar physiognomy or comparisons with NLRs, basically a guild approach. The subsequent assemblages then were analyzed statistically; structurally distinct assemblages

were found to correlate with different physical habitats. DiMichele and Phillips (1996) rescored Pennsylvanian-age plants according to a suite of ecological traits such as reproductive allocation, body size and form, and dispersal capability. They found that the phylogenetic signal could not be removed; Pennsylvanian wetlands were dominated by several widely distinct phylogenetic lineages, representing Linnean class and ordinal level groups. Thus, distinct body plans represent different fundamental approaches to being a plant and result in strong partitioning of ecological resources by clade. A similar pattern was found in the Middle to Late Devonian radiation of vascular plant classes (body plans) by DiMichele et al. (2001b), in which phylogenetic patterns, ecomorphic (functional type) distributional patterns, and ecological distributional patterns were found to overlap strongly, meaning that historical-phylogenetic patterns could not be removed except to separate ground cover from canopy and understory plants. Thus, functional-type categorizations of angiosperm-dominated systems appear to contain more convergences than those of the Paleozoic, where different kinds of ecosystems were dominated by phylogenetically widely disparate groups. This is an area where more examination is needed and one that should tell us much about evolution and the nature of ecological resource use and occupancy.

Integrative Studies

evolution and ecology

The conjunction of evolution and ecology has long been sought after. Yet, the two have long been practiced largely separately, despite such pithy characterizations as Hutchinson's (1965) ecological theater and evolutionary play, or Dobzhansky's (1973) classic observation that nothing in biology makes sense except in the light of evolution. Certainly, explicit linkage is more common in neobiology than in paleontology, if for no other reason than the vast differences in the number of practitioners. Nonetheless, because paleontologists are faced relentlessly with evolutionary and ecological history and their contingencies, there have been many attempts to bring them together at all scales from individual structural features to particular species to major groups.

Perhaps the best known example in paleobotany of the quest for an ecological explanation of evolutionary pattern is the origin of the angiosperms, Darwin's abominable mystery. In fact, this group is no more unheralded in its appearance than many of the major classical Linnean plant ordinal or class-level body-plan clades; there are just so many angiosperms around today to attract our attention. And, despite intensive study of seed-plant phylogeny over the past 20 years, a consistent, agreed-upon sequence of morphological transformations linking the angiosperms to the other seed plants has not been reached (e.g., Hilton & Bateman, 2006, for review). Thus, the angiosperms are representative, almost iconic, in fact, of an evolutionary problem—the origin of major morphological discontinuities. Explanations vary all over the conceptual map, and, still again, the subject is too complex for a comprehensive review here (see Feild & Arens, 2005). The implicitly gradualist hypothesis of a long-shrouded upland origin (Axelrod, 1952, 1972; Stebbins, 1965) visualized aridity as the driving selective force. A more sophisticated version of this model, making fewer assumptions about the tempo and mode of evolution and taking a more critical look at early angiosperm life histories, identified early angiosperms as weedy opportunists confined to river corridors, where they may have moved into areas of reduced competition in lowland settings from a place of origin in drier, marginal, extrabasinal habitats, also characterized by low competition (Hickey & Doyle, 1977). Taking a similar view, that physiological stress was the driving force of angiosperm morphology, but with an opposite viewpoint on where the selection occurred, Retallack and Dilcher (1981) put forth a coastal hypothesis, arguing that early angiosperms moved up from the coast rather than down from the uplands. In a modification of the upland weed model, early angiosperm evolution was hypothesized to have occurred directly within disturbed lowland habitats (Taylor & Hickey, 1992). Most recently, Feild and colleagues (Feild & Arens, 2004; Feild et al., 2004) have attempted to integrate physiological analyses of phylogenetically identified basal angiosperms (e.g., Feild et al., 2003a, b) with the fossil record. They suggest that disturbance and browsing by large vertebrates, specifically dinosaurs, in forest-shaded understories suppressed slower-growing ferns and gymnosperms and permitted rapidly growing, more physiologically escalated, angiosperms (sensu Vermeij, 1987) to make significant gains in resource occupancy. Labeling their hypothesis dark and disturbed, they note that the concept of key innovation in this instance is unlikely and that angiosperm dominance is a product of the overlap of a series of essentially unrelated factors.

Considerable evidence has accumulated from Late Paleozoic deposits, suggesting that novel body plans originate in areas of reduced competition, marginal to basinal settings, in effect lending credence to earlier models of upland (in reality, extrabasinal) origin of novelties, but without the presumption of long periods of gradual, microevolutionary change (recall the geological context, in this case the continental strati-

graphic record discussion, above). Most of this evidence comes from the appearance of scrappy, allochthonous remains of plants from derived clades (such as conifers, peltasperms, cycads, and corystosperms) in association with basinal wetland floras dominated by primitive lineages (such as lepidodendrid lycopsids, medullosan pteridosperms, marattialean tree ferns, and calamitean sphenopsids). For example, conifers first appear in the Middle Pennsylvanian, but only as bits and pieces of transported material, usually in basins proximate to tectonically active upland areas (Scott & Chaloner, 1983; Lyons & Darrah, 1989). Later floras rich in these xeromorphic plants occur in rare beds, intercalated within sequences otherwise characterized by dominance of wetland plants, with little overlap in taxonomic composition between these two kinds of floras. Ultimately, these initially extrabasinal, seasonally dry floras become the predominant vegetation in the basinal lowlands, correlating with directional changes in climate (Broutin et al., 1990; DiMichele & Aronson, 1992). The pattern repeats again as yet additional floras and vegetation types appear consisting of even more derived elements (DiMichele et al., 2001a, 2004; Kerp et al., 2006); in these examples, the derived plants often had been known only from much younger rocks, even of Mesozoic age, prior to their discovery in rare Paleozoic assemblages.

At the more detailed level of specific organisms, studies of fossil-plant biomechanics, when combined with stratigraphy and phylogenetic analyses, permit the evaluation of morphological trends in light of ecology. Examples, discussed earlier, include such things as the response of plant-growth architecture to wind shear via the evolution of stem structural safety factors (Niklas & Speck, 2001), or the filling of morphospace, considered in light of plant functional morphology (Niklas, 1999, 2000).

VEGETATIONAL RECOVERY FROM MEGADISTURBANCES

Studies of modern vegetational distribution along altitudinal and latitudinal gradients (Walter, 1985), and responses of past vegetation to climatic change during the Holocene and Pleistocene, demonstrate that plant distribution tracks climate and that plants respond to climatic changes rapidly (see reviews by Post, 2003; Parmesan, 2006). The boundaries of modern and past biomes may simply reflect the position and boundaries of major atmospheric circulation cells (Ziegler et al., 2003). In addition, there is evidence from the deep past that megadisturbances, such as the massive Cretaceous–Tertiary boundary bolide impact, had their greatest effects by changing climatic conditions, even if just for the short term and in locally variable ways. Furthermore, there are theoretical reasons to suspect that rapid changes in vegetational composition and structure could represent regime shifts, rapid changes from one stable compositional state to another once an environmental/climatic threshold is crossed (Scheffer & Carpenter, 2003; Ives & Carpenter, 2007).

The pre-Pleistocene record contains many well-studied examples of major changes in biotic composition in response to catastrophic (effectively instantaneous), rapid-directional, or prolonged-directional environmental change. The following are examples of some of the major events.

The onset of the Carboniferous ice age in the Mississippian is coincident with the development of widespread wetland floras and the demise of vegetation dominated by the most primitive seed plants (Pfefferkorn et al., 2000; Cleal & Thomas, 2005).

Major reorganization of tropical wetland vegetation of Euramerica occurred at the middle-late Pennsylvanian (Moscovian–Kasimovian) boundary, after millions of years of stability in the face of glacial-interglacial fluctuations (Pfefferkorn et al., 2000; DiMichele et al., 2002; Falcon-Lang, 2004), probably in response to rapid global warming (Phillips et al., 1974; Pfefferkorn & Thomson, 1982; Phillips & Peppers, 1984; DiMichele & Phillips, 1996; Cleal & Thomas, 2005). This may have been caused or exacerbated by tectonically driven elevational changes in Central Europe, which would have liberated large amounts of buried carbon and reduced the areas of carbon burial in the tropics (Cleal & Thomas, 2005). Middle Pennsylvanian–type wetland vegetation persisted through the Permian in China (Wang & Chen, 2001), where it remained very wet due to proximity to moisture sources and atmospheric circulation patterns (Ziegler, 1990). Dominance of weedy vegetation and a period of chaotic dynamics follow the Euramerican regional extirpations (Peppers, 1979, 1996; Pfefferkorn et al., 2008).

Vegetational changes in the dominant biome in most of equatorial Pangea occurred across the Carboniferous–Permian boundary (Kerp & Fichter, 1985; Broutin et al., 1990; DiMichele & Aronson, 1992) in response to long-term changes in temperature and rainfall patterns, again perhaps linked to changes in the extent of glacial ice and atmospheric CO_2 (Montañez et al., 2007).

Terrestrial and marine biotic changes near the Permian–Triassic boundary, in response to complex causation (Erwin, 1993), resulted in long pseudosuccessional recovery lags and wholesale vegetational restructuring (Looy et al., 1999), with evidence of persistence of many forms in extrabasinal areas (Kerp et al., 2006).

Major vegetational restructuring and species turnover occurred at high latitudes (and possibly globally) at the Triassic–Jurassic boundary (McElwain et al., 1999) apparently caused by a rapid 3%–4% increase in atmospheric CO_2.

The Cretaceous–Tertiary boundary had profound effects on terrestrial vegetation globally. Initially debated with regard to extent and rapidity of plant response (Hickey, 1981; Tschudy, 1984), it is now clearly documented to be rapid (Johnson, 1992) and global, but with variation dependent on local and regional climate and topography (Wilf & Johnson, 2004). Followed by what may have been a pseudo-succession similar to that of the early Triassic (Wolfe & Upchurch, 1986, 1987), like that former event, it appears that lowlands may have been more homogenous than was characteristic of extrabasinal areas, indicated by rare finds of distinct and diverse floras (Johnson & Ellis, 2002). Significantly different interactions between plants and animals may have ensued, delaying the radiation of large mammals for millions of years (Wing & Tiffney, 1987).

A short, extreme pulse of global warming at the Paleocene–Eocene boundary, the so-called Paleocene–Eocene thermal maximum (PETM), was driven by rapid global warming, which correlates with sharp changes in carbon isotopic composition of marine sediments (Zachos et al., 2005). Vegetational response was rapid and accompanied by large range shifts (Wilf, 2000; Wing et al., 2005). Finally, long-term climatic cooling occurred from the late Eocene through the early Oligocene (see papers in Terry & Evanoff, 2006) and was accompanied by vegetational changes that effectively tracked the changes in climate (e.g., Jaramillo et al., 2006).

Although each of these changes is unique, comprising different taxonomic compositions and different causes, either in kind or in degree, and occurring under different continental configurations and prevailing climates, there are unmistakable similarities. First, all confirm that vegetation responds rapidly to environmental changes, be those changes in extremes or seasonal distribution of temperature or rainfall. Long-term climatic changes demonstrate that plants, particularly if considered at the scale of biomes or large-scale species pools, track climate relatively closely and faithfully. Often, this is accompanied by intrabiomic changes in dominance-diversity while retaining much of the basic species-pool presence-absence composition. Rapid to catastrophic changes are almost always accompanied by dominance of opportunistic weedy vegetation during the initial response. In some instances, this vegetation gives rise to persistent dominant forms, though, in most cases, it is rapidly replaced by more K-selected growth forms. Pseudo-successions follow several of these events; however, there are indications from occurrences of rare intercalated or precocious floras that more complex and diverse vegetation continued to persist or exist refugially in extrabasinal areas. Thus, such pseudo-successions would be most indicative of long-lasting environmental disruption of the lowland wetlands and inhibition of reestablishment of most surviving lineages from surrounding areas. Finally, these events indicate that plants and vegetation can and do survive major environmental changes, but that recovery from these disruptions generally takes millions of years (e.g., plants typical of Mississippian seasonally dry habitats reappear in similar habitats during the late Pennsylvanian and Permian [Mamay & Bateman, 1991; Mamay, 1992]; plants of Mesozoic habitats appear precociously in the Paleozoic [DiMichele et al., 2001a; Kerp et al., 2006]; dominant conifers both occur precociously in the Pennsylvanian and survive the Permo–Triassic extinction though returning after a long lag [Lyons & Darrah, 1989; Looy et al., 1999]). It must be reiterated that apparent stratigraphic lags in the occurrence of taxa are impacted by the regional and basinal processes operating on preservation potential of organic matter (see sequence stratigraphic discussion above; Gastaldo et al., 2005).

PLANT-ANIMAL INTERACTIONS

Our coverage of this expanding field of paleoecology will be abbreviated because most of the research focuses on animal paleobiology in which the plants are substrates for animal activities. There are important areas where the ecological attributes of the plants and animals are considered jointly, generally involving comparative phylogenetic patterns.

Insect-mediated pollination biology of angiosperms (and potentially other groups of plants, though there is greater speculation there) has been an area where some debate has arisen due to potential conflicts between theory and data. Crepet (1979, 1985) suggested that the coevolution of angiosperms and faithful insect pollination during the Cretaceous was a powerful driving force both in the evolution of the flowering plants and of their pollinators. Many modern students of insect systematics and ecology have suggested that the expansion of the flowering plants in fact created a substrate for the tremendous radiation of the insects. Labandeira and Sepkoski (1993), in an analysis of the fossil history of insect diversity and timing of origination of functional feeding groups, found that insects radiated significantly earlier than the angiosperms, during the Triassic and Jurassic, and argued for the decoupling

of insect diversification from that of flowering plants. Grimaldi (1999) has challenged this interpretation of no relationship, considering conjointly the fossil record of the major groups of insect pollinators and phylogenies of these clades. He argues that there is strong coincidence in the timing of insect and entomophilous angiosperm radiations. This debate continues (e.g., Labandeira, 2002).

Much of the literature on plant-arthropod interactions has focused on the trace-fossil record of arthropod activity. Most evidence from the earliest land plants suggests that arthropods were acting mainly as detritivores, with only limited evidence for attacks on living plants (Kevan et al., 1975; Labandeira, 1998). By the Carboniferous, there is considerably more evidence for arthropod predation on living plants. The majority of data, such as that from coprolites (Scott, 1977b; Labandeira et al., 1997), suggests that detritivory remained the major way in which plant productivity entered the invertebrate food chain (Scott & Taylor, 1983; Scott et al., 1985). There is some indication that insects were involved in medullosan pollination, based on coprolite data (Scott & Taylor, 1983). Labandeira (2006a) reports that the earliest evidence of external foliage feeding occurs in the Late Mississippian, and that insect herbivory is concentrated particularly on medullosan pteridosperms in tropical floras, also noted by Scott et al. (1992), and on glossopterid seed plants in the Southern Hemisphere. Evidence of distinct forms of feeding, such as piercing and sucking, has also been documented in the Pennsylvanian (Labandeira & Phillips, 2002). During the Permian, in tropical regions, it appears that insect feeding was also concentrated preferentially on certain clades (Beck & Labandeira, 1998; Labandeira & Allen, 2007). Labandeira (2006a) finds two further expansions of insect herbivory, one beginning in the early Mesozoic, and the other taking place in the mid-Early Cretaceous, coincident with the evolutionary radiation of flowering plants. Insects seem to have established modern food-web patterns by the end of the Carboniferous, with the full spectrum of insect functional feeding groups active by the end of the Triassic (Labandeira, 2006b).

The record of vertebrate-plant interaction is more difficult to interpret. Not only might it involve herbivory (Chin & Gill, 1996), which will be considerably more difficult to detect from feeding traces on plants than is the case for arthropods, and detritivory (Chin, 2007), but vertebrates, particularly those of large size, can considerably alter ecosystems through various kinds of disturbance (e.g., transformation of vegetational structure by elephants; Wing & Buss, 1970). The earliest evidence of vertebrate herbivory is from the later Carboniferous (Hotton et al., 1996; Sues & Reisz, 1998). Interestingly, these early occurrences are not followed by a major radiation of vertebrate herbivores. Based on patterns of taxonomic diversity, there is a diversification lag paralleled by the evolution of herbivorous feeding strategies in insects (Labandeira, 2006a). Some of these early vertebrates had large bodies and small heads, suggesting that they may have been fermentative gut processors. The first vertebrate with indisputable evidence for oral processing of tough, high-fiber plant material is of Late Permian age from Russia (Rybczynski & Reisz, 2001). Prior to the Late Permian, vertebrate communities are dominated by what appear to be carnivorous forms. It is not until the Late Permian that trophic pyramids appear similar to those of modern communities (DiMichele & Hook, 1992; Sues & Reisz, 1998). Many Late Paleozoic seeds are large and have fleshy seed coats; Tiffney (2004) suggests that these fleshy seeds may have been consumed and dispersed by vertebrates, which may have confused them for carrion. However, he also concludes that there is, overall, relatively limited evidence for vertebrate dispersal of most Paleozoic seeds (but recall the disparity of preservational requirements between plant and vertebrate remains).

A deep understanding of the effects of dinosaurs on Mesozoic plant communities has been elusive. It is clear that these large animals would have been major agents of mechanical disturbance and also consumed, probably in an unspecialized manner, a wide spectrum of plants (Coe et al., 1987; Tiffney, 1997; Chin & Kirkland, 1998; Chin, 2007). In addition, they may have been major agents in promoting the expansion of weedy angiosperms (Feild & Arens, 2005). Coevolution with dinosaurs also may have had selective effects that can still be seen today on the chemical and morphological attributes of clades that reached their zenith in the Mesozoic, such as cycads. Many of these plants contain neurotoxins in their leaves, which may have deterred vertebrate herbivory, but not in the (edible) sarcotestas of their seeds, permitting seed herbivory and dispersal (Mustoe, 2007).

Following the demise of the dinosaurs at the end of the Cretaceous, large areas of forest developed in the Paleocene, coincident with a delayed appearance of large-bodied mammalian vertebrates (Collinson & Hooker, 1991); Wing and Tiffney (1987) attributed this suppression of vertebrate body size increase to the vegetational structure. Tiffney (2004), in a review of the role of vertebrates in seed-plant dispersal through time, goes so far as to suggest that only with the demise of the dinosaurs could the angiosperm-bird-mammal dynamic evolve, resulting in a fundamental change in ecological dynamics between the Mesozoic

and Cenozoic. We may presume that Tertiary ecosystems, especially those of the post-Paleocene, functioned much like those of the present with regard to the variety of plant-vertebrate interactions (see the reviews by Collinson & Hooker [1991], Wing [1998], and Tiffney [2004], which bring together much of the literature on vertebrate interaction with plants).

ECOLOGICAL ASSEMBLY RULES IN SPACE AND TIME

The concept of assembly rules in ecology has been debated with varying intensity and in various guises nearly throughout the history of the discipline. The very idea of ecological assembly implies determinism and predictability of outcome—it is the nature of this determinism and the particular nature of the expectations about outcome that lie at, or close to, the root of this long-term controversy. From the time of the long-running Clementsian superorganism (Clements, 1916) versus Gleasonian species individualism (Gleason, 1926) debate over succession and climax communities, to more modern incarnations of this discussion about the degree of interaction and coevolution in communities (e.g., Diamond, 1975; Conner & Simberloff, 1979), to recent more generalized formulations (Weiher & Keddy, 1999), the inference of rules of community assembly, drawn empirically from time-space patterns, persists despite general skepticism (e.g., Foster et al., 1990). General skepticism is drawn from things such as the responses of plants to the retreat of ice following the last glaciation (e.g., Overpeck et al., 1992; Jackson, 2000), or studies of short- and long-term, apparently stochastic, changes in the composition of ecological communities (Hubbell & Foster, 1986). Hubbell's (2001) formulation of a general theory of ecological dynamics, in which species are treated neutrally as identical elementary particles, changes the terms of the debate to a great degree, in that deterministic processes may lead to a variety of outcomes. Furthermore, the explicit consideration of historical constraints on modern ecological patterns, via inclusion of phylogenetics and fossil history (Webb et al., 2006), into the explanatory framework, impinges on the matter of assembly because past events may have impacts far into the future, a basic tenet of assembly-rules considerations.

In deep-time paleontology, there has been little explicit consideration of the matter of ecological assembly rules, primarily because of the need for a sequence of fossil deposits over a specific time interval and covering a spatial extent that would permit evaluation of patterns of species addition sequences and combination patterns to be rigorously evaluated statistically. However, it is the interactions of populations and individuals that dictate and scale upward into larger ecological patterns (e.g., Hubbell, 2001); surely, such interactions are what also underlie the dynamics of evolution. Thus, it should be expected that evolutionary assembly of vegetation over long periods of time should be under the same basic controls as assembly in space over short periods of time. The most general models for controls on community assembly are summarized by Belyea and Lancaster (1999) and Weiher and Keddy (1999). Using somewhat different jargon, these authors present basically the same hierarchical model, consisting of three components. The model also applies to evolutionary assembly over long spans of time as it does to spatial assembly on post-glacial time frames. The following factors control the composition of any site-specific assemblage. (1) Can a species get to the site? In other words, is it a member of the regional species pool, or "metacommunity" (sensu, Holyoak et al., 2005)? (2) Once on a site, can the species survive under the local physical conditions? Does it have the physiological and structural capabilities to exist under the soil edaphic conditions, the local aspect, and the light regime of the site? (3) If the first two criteria are met, can the representatives of the species outcompete representatives of other species already on the site, or those that arrive later? To a certain degree, this subsumes and may largely be a consequence of the effects of incumbency, which are related to population size, likelihood of expropriating resources, likelihood of colonizing unutilized or underutilized resources (e.g., Gilinsky & Bambach, 1987), and fecundity.

The most explicit statement of a model for evolutionary assembly of ecological systems is that of Valentine (1980). This heuristic model describes an adaptive zone as a checkerboard, or a pattern of tesserae through time. Each square represents an ecological niche. The initial population to establish within this landscape finds little or no competition for resources and can survive even if only marginally able to live on the site. Given that most speciation will produce descendants similar to the ancestor, ecological space is gradually filled by new species around the resource space of the initial occupant. Coincident with small-scale evolution are rare larger-scale departures in ancestor-descendant similarity, most of which produce nonviable offspring, but some of which will produce forms that can survive if they can reach and survive in resource spaces with low or no competition. Gradual filling of ecological resource space then begins around these new resource nodes. Eventually, as ecological resource space fills, large-scale departures decline in frequency because their chances of survival decline. This is due to the effects of incumbency within the overall adaptive landscape.

Walker and Valentine (1984) later criticized this model because of the supposed lack of niche saturation in ecospace (see Patzkowsky & Holland [2003] for a similar paleontological perspective), a matter widely addressed in the neoecological literature. In addition, the static view of niche space comports neither with the way in which niche generally is conceived (Whittaker et al., 1973), that is as defined by species rather than as preexisting, nor with the similar, expanded view, known as niche construction (Odling-Smee et al., 1996), which argues that organisms, by their actions and interactions, create and expand niche/resource space. Nonetheless, the Valentine (1980) model, though in need of modification, is still a strong basic framework for uniting evolutionary models of ecological assembly with mainly spatial neoecological models. DiMichele et al. (2001b, 2005) have applied some aspects of these models to the examination of ecological assembly patterns in Paleozoic plant communities.

An entirely different way of examining ecological assembly and the rules by which plant assemblages may be structured is emerging from the study of plant allometry. In short, beginning with metabolic scaling theory (West et al., 1997; Brown et al., 2004), a straightforward set of linkages has been developed among plant size, mass, metabolic rate, etc., all the way to spatial-density aspects of populations and mixed-species stands (Enquist et al., 2002, 2003, 2007; Niklas et al., 2003; Niklas, 2006). In the model from West et al. (1997), the metabolic rate of an organism scales to its body mass raised to the 3/4 power. These relationships have been found to apply to the scaling of plant-body size within populations, and from that to entire multispecies plant communities (Niklas & Enquist, 2001; Niklas et al., 2003). In its most fully elaborated form (Enquist et al., 2007), these scaling laws permit basic physiological attributes of organisms to be linked directly to the ecological structure of plant communities in a "taxon-free" manner, given that the basic rules appear to apply to all major groups of eukaryotic photoautotrophs (Niklas, 2006). The strength of this approach, to quote Enquist et al. (2007) "is that it reduces much of the complexity of organisms and ecosystems to simple, but universally applicable, physical and chemical principles." In certain ways, this approach meshes with Hubbell's (2001) neutral theory, in that the particulars of species composition do not really matter to ecosystem functioning. Where the fossil record permits (e.g., some of the examples given in Enquist et al. [2007]), the predictions about organism size and stand densities can be tested directly, often in systems for which there is no modern equivalent. Conversely, to the extent that these scaling generalities are proven to hold (also consider the leaf physiological rate spectrum, discussed above), they may be useful to allow estimation of attributes of ancient systems. The degree to which this approach becomes central to ecology remains to be seen.

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Erratum

Watanabe, K., T. Yahara, G. Hashimoto, Y. Nagatani, A. Soejima, T. Kawahara & M. Nakazawa. 2007. Chromosome numbers and karyotypes in Asteraceae. Ann. Missouri Bot. Gard. 94: 643–654.

In Appendix 1 on p. 653, under Eupatorieae, the second accession for *Chromolaena pedunculosa* (Hook. & Arn.) R. M. King & H. Rob. was erroneously listed as $2n = 20II + 10I$. The correct accession is $2n = 10II + 10I$. The authors regret the error.

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ASSUMPTION 0 ANALYSIS: COMPARATIVE PHYLOGENETIC STUDIES IN THE AGE OF COMPLEXITY[1, 2, 3]

Daniel R. Brooks[4] *and Marco G. P. van Veller*[5]

ABSTRACT

Darwin's panoramic view of biology encompassed two metaphors: the phylogenetic tree, pointing to relatively linear (and divergent) complexity, and the tangled bank, pointing to reticulated (and convergent) complexity. The emergence of phylogenetic systematics half a century ago made it possible to investigate linear complexity in biology. Assumption 0, first proposed in 1986, is not needed for cases of simple evolutionary patterns, but must be invoked when there are complex evolutionary patterns whose hallmark is reticulated relationships. A corollary of Assumption 0, the duplication convention, was proposed in 1990, permitting standard phylogenetic systematic ontology to be used in discovering reticulated evolutionary histories. In 2004, a new algorithm, phylogenetic analysis for comparing trees (PACT), was developed specifically for use in analyses invoking Assumption 0. PACT can help discern complex evolutionary explanations for historical biogeographical, coevolutionary, phylogenetic, and tokogenetic processes.

Key words: Assumption 0, biogeography, cladogeny, coevolution, evolutionary complexity, horizontal transfer, hybridization, PACT, phylogenetic analysis for comparing trees, phylogenetic systematics, phylogeography, reticulation, taxon pulse, tokogeny.

[1] This and the three articles that follow it are the proceedings of the 52nd Annual Systematics Symposium of the Missouri Botanical Garden, "Reconstructing Complex Evolutionary Histories: Gene-Species Trees, Historical Biogeography, and Coevolution." The symposium was held 7–9 October 2005, at the Missouri Botanical Garden in St. Louis, Missouri, U.S.A.

[2] This was the 50th Missouri Botanical Garden Annual Systematics Symposium to be supported by a grant from the National Science Foundation (grant DEB-0515933). The meeting was organized and moderated by Richard Mayden of Saint Louis University; P. Mick Richardson was responsible for the smooth running of the symposium, with the welcome and able assistance of Alina Freire-Fierro, Mary McNamara, Sandy Lopez, Donna Rodgers, William Guy, and Zubin Chandran; Victoria C. Hollowell, Beth Parada, and Allison Brock (Missouri Botanical Garden Press) were responsible for the publication of the symposium proceedings.

[3] This contribution is based on a presentation made by DRB at the 2005 Annual Systematics Symposium of the Missouri Botanical Garden. We thank Rick Mayden for the invitation to participate in that symposium, as well as Peter Raven, Mick Richardson, and the other organizers of the symposium. We also express special appreciation to Marco Roos, E. O. Wiley, and Rino Zandee, who first articulated Assumption 0. DRB acknowledges funding from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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The universe is structured by laws, and science is the search for methods of data analysis and theories providing powerful general explanations, all in terms of those general laws. This ontology of simplicity has guided the development of western science for nearly 2500 years, embodied in the principle of parsimony (Latin "parcere," to spare). Aristotle (350 B.C.E.) articulated the ontological view of the principle of parsimony, the postulate that "nature operates in the shortest way possible" and "the more limited, if adequate, is always preferable." The principle of parsimony is also linked with the English philosopher and Franciscan monk William of Ockham (ca. 1285–1349), who advocated the use of what is known as "Ockham's razor": "pluralitas non est ponenda sine neccesitate" ("plurality should not be posited without necessity") and "non sunt multiplicanda entia praeter necessitatem" ("entities should not be multiplied unnecessarily"). In this sense, the principle of simplicity obliges us to favor theories or hypotheses that make the fewest unwarranted, or ad hoc, assumptions about the data from which they are derived. This epistemological use of the principle does not necessarily imply that nature itself is parsimonious. Indeed, despite the best efforts of philosophers for more than 700 years, no link between parsimony and truth has ever been established. Nonetheless, most scientists conduct their research as if they believe that nature is parsimonious in some sense and, as a consequence, they place much more credence in simple than in complex theories.

The cosmologist Stephen Hawking has dubbed the 21st century the century of complexity, leading a parade of physicists who began to think about ontological complexity in the latter decade of the 20th century. Biology should be well poised to take advantage of this sea change in our understanding of the nature of science and the universe, having discovered an ontology of complexity called Darwinism almost 150 years ago. Few fully realize that Darwinism is not a simple theory:

> "... there are two factors: namely, the nature of the organism and the nature of the conditions. The former seems to be much more the important; for nearly similar variations sometimes arise under, as far as we can judge, dissimilar conditions; and, on the other hand, dissimilar variations arise under conditions which appear to be nearly uniform."
>
> —Darwin (1872: 32)

Most evolutionary biologists consider this passage no more than a general repudiation of Lamarckism. We believe, however, that it is one of the first modern articulations of a complex theory: Darwin proposed that evolution is an emergent property of asymmetrical interactions between two different causal agents, each with its own properties, producing outcomes that are not readily predictable from knowledge of the properties of either agent and, thus, historically contingent.

Darwin thought that organisms were historically and developmentally cohesive wholes, and it was in the "nature of the organism" to produce offspring that were all highly similar (but not identical) to each other and to their parents and other ancestors. He also postulated that reproduction produced variation without regard for environmental conditions, and it was in the "nature of the organism" to produce these offspring in numbers far exceeding the resources available for their support. When this inherent overproduction produced variety in critical characters, natural selection would preserve the versions that were functionally superior in that particular environmental context (adaptations). Whenever an environment changed, those organisms that already had the adaptations necessary to survive would do so, whereas those lacking appropriate adaptations would not. The production of organismal diversity thus required that organisms be at once autonomous from, and sensitive to, the environment, another example of complexity.

Darwin visualized his viewpoint about the complexity of evolution with two metaphors, the phylogenetic tree and the tangled bank. The phylogenetic tree points to complexity arising from irreversible phenomena. By referring to species as "communities of descent" and placing them in a single "tree of life," Darwin emphasized that the fundamental explanatory principle is shared history. The tangled bank, by contrast, points to complexity arising from biological associations that do not share the same temporal and spatial origins, producing reticulated (tangled) patterns of descent and association.

By the end of the 19th century, most biologists had adopted the view that evolution had occurred, but many were uncomfortable with Darwinism's lack of simple general laws, and the discovery of the "Laws of Inheritance" by Mendel did little to quell this unrest. Bowler (1983) documented the eclipse of Darwinism during the period of 1890–1940. The most influential theories during that time were neo-Lamarckism and orthogenesis, both theories of simplicity. As a theory of environmental determinism, neo-Lamarckism placed causality solely in the nature of the conditions. Orthogeneticists, by contrast, asserted that the environment played little or no role in evolution, thereby placing causality solely in the nature of the organism. The eclipse of Darwinism is assumed to have ended with the emergence of neo-Darwinism in the 1930s. Neo-Darwinism focused on Darwin's key mechanism, natural selection. Buoyed by advances in understanding the mechanisms of genetic inheritance

and some elegant experiments, this became the dominant theory for evolutionary biologists during the second half of the 20th century. Some founders fully expected neo-Darwinism to encompass the panorama of phenomena encompassed in Darwin's original formulation:

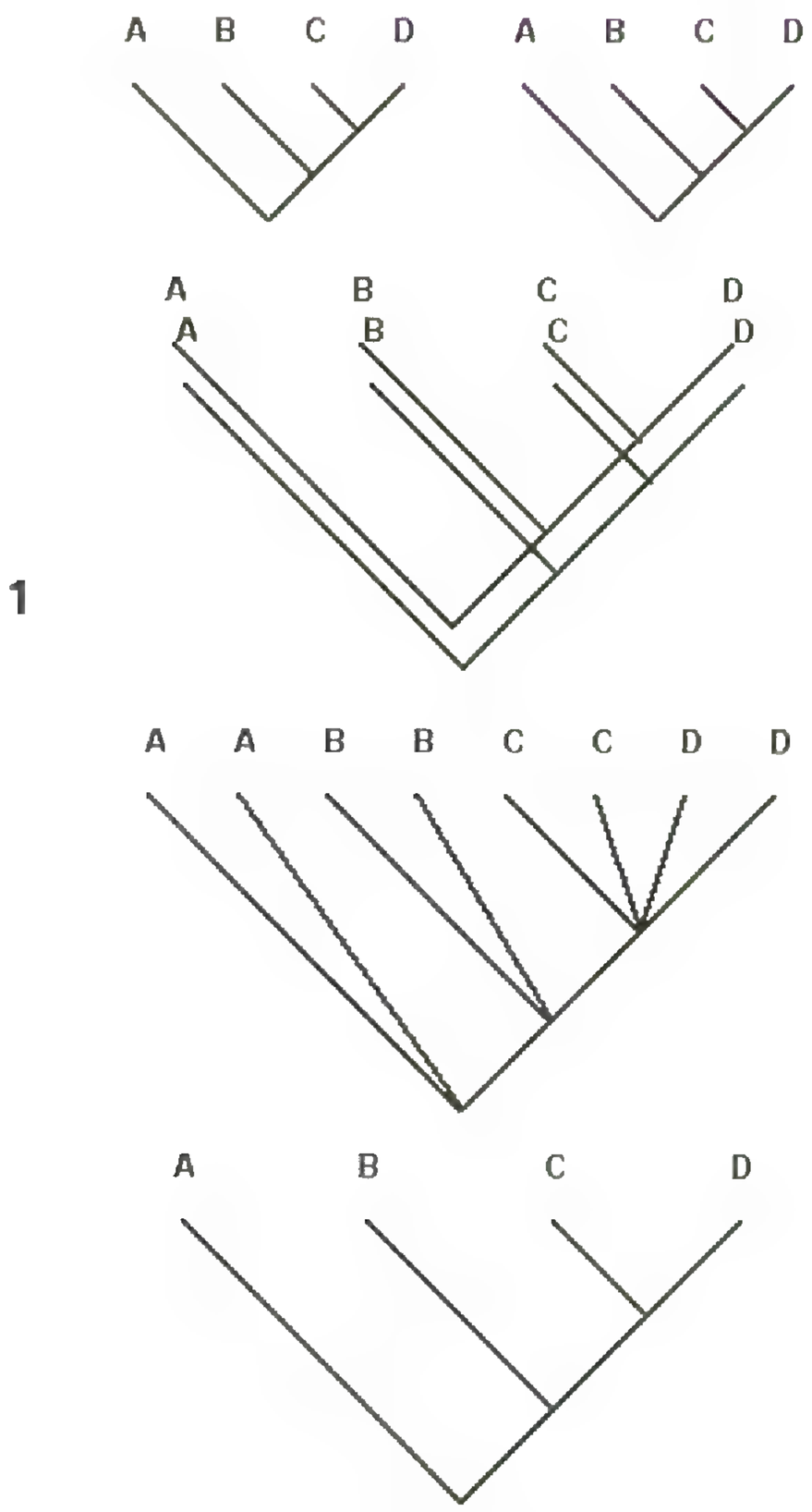
> "...in every part of the whole, wonderful history of life, all the modes and all the factors of evolution are inextricably interwoven. The total process cannot be made simple, but it can be analyzed in part. It is not understood in all its appalling intricacy, but some understanding is in our grasp, and we may trust our own powers to obtain more."
>
> —Simpson (1953: 393)

Sadly, this was not to be the case. As noted by Gould (1983) using the rubric "the hardening of the synthesis," during the latter third of the 20th century, neo-Darwinism maintained a narrow focus on population genetics, acknowledging other aspects of evolution but asserting that all of them were manifestations of population genetics and natural selection and, therefore, all appearances of complexity could be reduced to the sometimes computationally complex, yet ontologically simple, principles of population genetics. Like the neo-Lamarckians, neo-Darwinians focused their attention on the environmental conditions correlated with the survival of organisms, populations, and species. Thus, many explanations expounded by neo-Darwinians sound Lamarckian because the nature of the organism has been reduced to almost nothing. For many today, the nature of the organism is to be a blind watchmaker made up of selfish genes. Organisms exist, but they have no "nature" autonomous from their surroundings. This produces a theory that is parsimonious, yet lacking Darwin's panoramic view of biological diversity.

During the past 25 years, a growing number of scientists and philosophers have called for modifications of evolutionary theory (e.g., Eldredge, 1985, 1986, 1989; Brooks & Wiley, 1986, 1988; Maynard Smith & Szathmary, 1995; Brooks, 1998, 2000, 2001, 2002; Collier & Hooker, 1999; Brooks & McLennan, 2000 and references therein). Many of these calls seek to reintroduce Darwin's postulates about "the nature of the organism" and the "nature of the conditions" to develop analytical methods for integrating the tree of life and the tangled bank metaphors, and to establish fundamental guiding principles or null hypotheses for complex evolutionary phenomena occurring on different spatial and temporal scales.

The phylogenetics revolution that began in the late 1960s (Hennig, 1966) and became entrenched beginning in the 1980s (for a review, see Brooks & McLennan, 2002) produced quantitative analytical methods for effectively documenting the complexity of the tree of life. Phylogeneticists discovered quickly that most phylogenetic tree complexity is manifested in a relatively small number of linearly organized historical correlations (which we consider to be the communities of descent) and, therefore, is (once again) computationally complex but ontologically simple. Methods developed for analyzing historical associations described by tangled bank complexity assumed that such associations could also be adequately described and explained using phylogenetic tree–based reasoning (e.g., Brooks, 1985). Tangled bank complexity, however, produces reticulated historical relationships in addition to the relatively simple linear ones. We now know that methods of analysis based on phylogenetic tree–based reasoning produce internally inconsistent results in direct proportion to the degree of tangled bank complexity in the data, an indication that those methods have oversimplified the data (van Veller et al., 1999, 2000, 2001, 2002, 2003; Dowling, 2002; Dowling et al., 2003). Wojcicki and Brooks (2004, 2005) developed an algorithm called phylogenetic analysis for comparing trees (PACT) that permits phylogenetic systematic principles to be used in describing tangled bank type complexity.

PACT is based on the assumption that tangled bank complexity is based on individual parts that each conform to relatively simple tree-like complexity which, when combined, may nonetheless produce complex patterns of both general and unique relationships, including the possibility of reticulated relationships. PACT uses three guiding principles: (1) Assumption 0 (Wiley, 1986, 1988a, b; Zandee & Roos, 1987): in order to not oversimplify, you must analyze all input data without modification, and your final result must be logically consistent with all input data; (2) the duplication rule (Brooks & McLennan, 2002): Assumption 0 is violated with analyses by methods of simplicity when the data result from reticulated processes. Oversimplification can be avoided (i.e., Assumption 0 can be satisfied) in such cases by duplicating entities with reticulated histories; and (3) epistemological parsimony (Brooks & McLennan, 2002): in order to find the maximum number of generalities supported by the data, entities should not be duplicated beyond necessity. Therefore, we should make only enough duplications to satisfy Assumption 0. Underscoring its connection with the ontology of complexity, note that Assumption 0 is more fundamental than parsimony. If evolutionary patterns were simple, Assumption 0 would never be violated, so we would never need to duplicate entities and would never need parsimony. What we are seeking is the most general explanation possible



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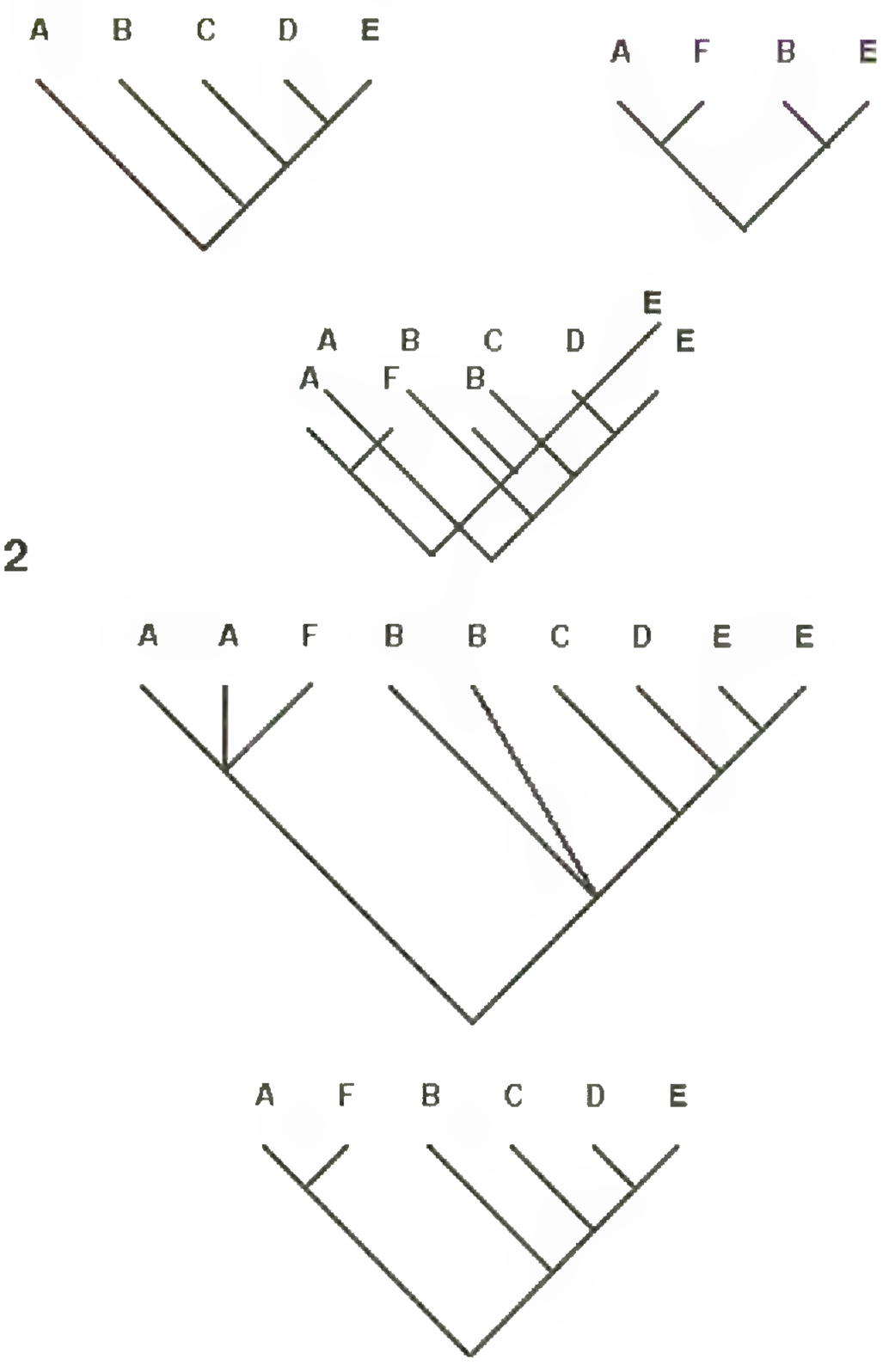

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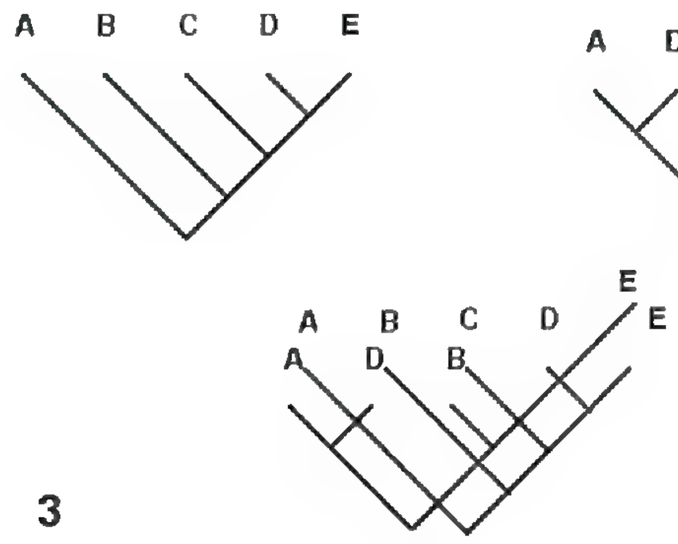
Figures 1 and 2. —1. PACT analysis for simple congruence. Top = two hierarchical patterns based on different data but pertaining to entities A, B, C, and D. Second from top = the two hierarchical patterns aligned by common elements. Third from top = both hierarchical patterns superimposed on each other. Bottom = PACT solution based on applying combination rule 1 (Y + Y = Y). —2. PACT analysis for two incongruent patterns. Top = two hierarchical patterns based on different data but pertaining to entities A, B, C, D, E, and F. Second from top = the two hierarchical patterns aligned by common elements. Third from top = both hierarchical patterns superimposed on each other. Bottom = PACT solution based on applying combination rule 2 (Y + YN = YN). Y(A) + YN (AF) = YN (AF); Y (E) + YN (ED) = YN (ED); Y (B) + YN (C(DE)) = YN (B(C(DE))).

given the data (i.e., the ontology of complexity), not the simplest explanation we can imagine (i.e., the ontology of simplicity) (see van Veller & Brooks, 2001; Brooks & McLennan, 2002). What we have to guard against is the use of models and analytical methods that oversimplify the data in an effort to generalize. That is, simplicity is not always the most parsimonious depiction of the real world (van Veller & Brooks, 2001).

PACT utilizes any data that can be represented as hierarchical strings, ranging from gene trees and other character state trees to species phylogenies. Wojcicki and Brooks (2004, 2005) recognized that nested (historical) hierarchical patterns in nature are combinations of only six different classes of pattern modules. Analysis of a set of data is accomplished by selecting any one of the input data strings as the initial template. Next, the final analysis is built by sequentially aligning each additional data string with the template. Thereafter, combinations are made to satisfy Assumption 0 using four combination rules. The combination rules are illustrated in Figures 1–6 and explained briefly in the figure legends; for more details, see Wojcicki and Brooks (2004, 2005). Some workers seem to have assumed that PACT is an algorithm for the method called Secondary BPA (Brooks & McLennan, 2002). A purported critique of PACT by Salvador Arias et al. (2008) addresses shortcomings of secondary BPA overcome by PACT that were specifically addressed by Wojcicki and Brooks (2004, 2005).

Elucidating complex evolutionary patterns using PACT comes with a cost of sorts. Explaining the complexity of a set of observations can only be achieved by abandoning a priori expectations of mechanism. For example, is the presence of a species in area F in Figure 2 an indication of a unique event involving the production of a novelty in one of the data strings, or is it an indication of a unique event involving loss from the other? PACT produces the same result regardless of the interpretation, so no assertions or assumptions about processes are em-



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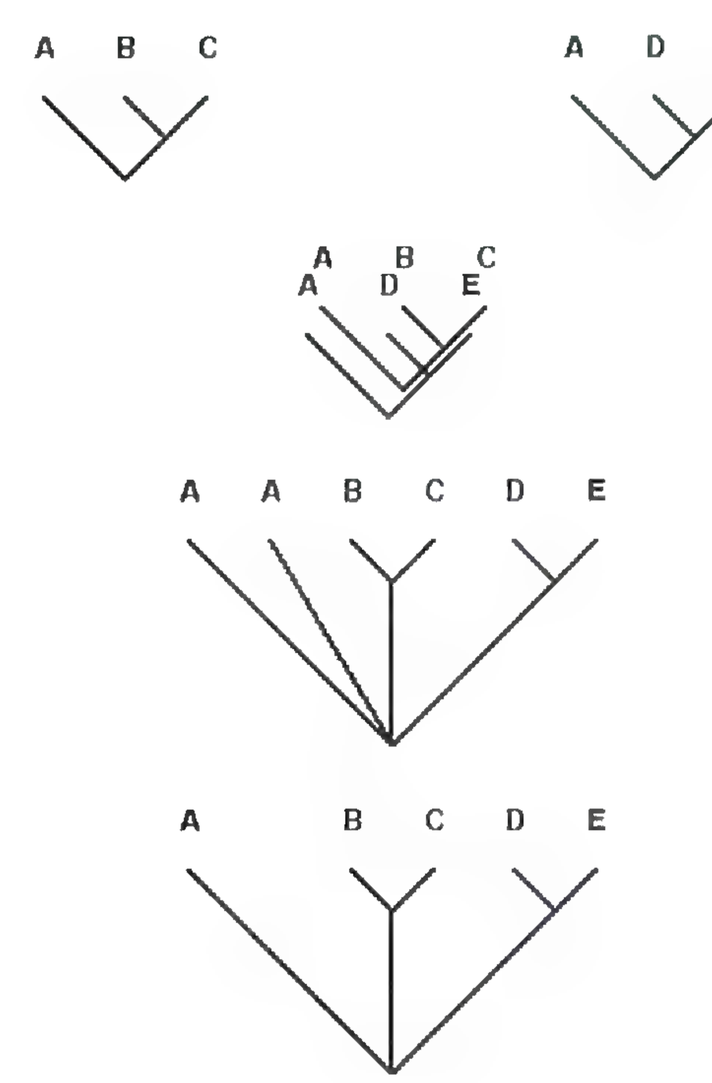

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Figures 3 and 4. —3. PACT analysis for two incongruent patterns. Top = two hierarchical patterns based on different data but pertaining to entities A, B, C, D, and E. Second from top = the two hierarchical patterns aligned by common elements. Third from top = both hierarchical patterns superimposed on each other. Bottom = PACT solution based on applying combination rule 2 (Y + YN = YN). Y (A) + YN (AD) = YN (AD); Y (E) + YN (ED) = YN (ED); Y (B) + N (C(DE)) = YN (B(C(DE))). Note that this example is precisely the same as the one shown in Figure 2, with the exception that in this case we find evidence of reticulated relationships. —4. PACT analysis for two congruent patterns. Top = two hierarchical patterns based on different data but pertaining to entities A, B, C, D, and E. Second from top = the two hierarchical patterns aligned by common elements. Third from top = both hierarchical patterns superimposed on each other. Bottom = PACT solution based on applying combination rule 3 (YN + YN = YNN). Y (A)N (BC) + Y (A) N (DE)) = YNN (A(BC)(DE)). (One should not over-interpret the data.)

bedded in the algorithm. Similar reasoning can be applied to concepts such as lineage duplication, lineage sorting, and ancestral areas/hosts. All these concepts pertain to hypotheses of mechanism, and in order to maximize empirical robustness and avoid the possibility of circularity, all processes should be inferred following an analysis of data, not built into it.

It is for this reason that PACT makes combinations beginning with the youngest members of the data strings being compared. This avoids the assumptions, unintentionally embedded in all previous methods for historical biogeography and coevolution, that (1) all species associated today originated together at the same place and time; and (2) when there is ambiguity, we resolve the ambiguity beginning with the oldest (most basal) portions, amounting to an unacceptable assumption that the older the evolutionary event, the more accurate our information about it.

PACT analysis is, therefore, only the beginning of a study, not an end in itself. Explaining the result of a PACT analysis requires reference to guiding principles that are more comprehensive than those currently in use. We illustrate this briefly for four different research programs in comparative evolutionary biology.

Research Programs

historical biogeography

Biogeography is the basis for understanding the origin of species, the assemblage of communities, and the differential radiation of clades (Brooks & McLennan, 2002). In the latter third of the 20th century, historical biogeography produced two simple and elegant theories that were based on an ontology of simplicity rather than complexity.

The first of these was the equilibrium theory of island biogeography (ETIB) (MacArthur & Wilson, 1963, 1967). This theory is based on the view that dispersal from "source" areas to "islands" (actual or metaphorical), mediated by island size and distance, produces linear log-normal species-area relationships. Noise in the system or the effects of contingency comprise in situ speciation and extinction. From this,

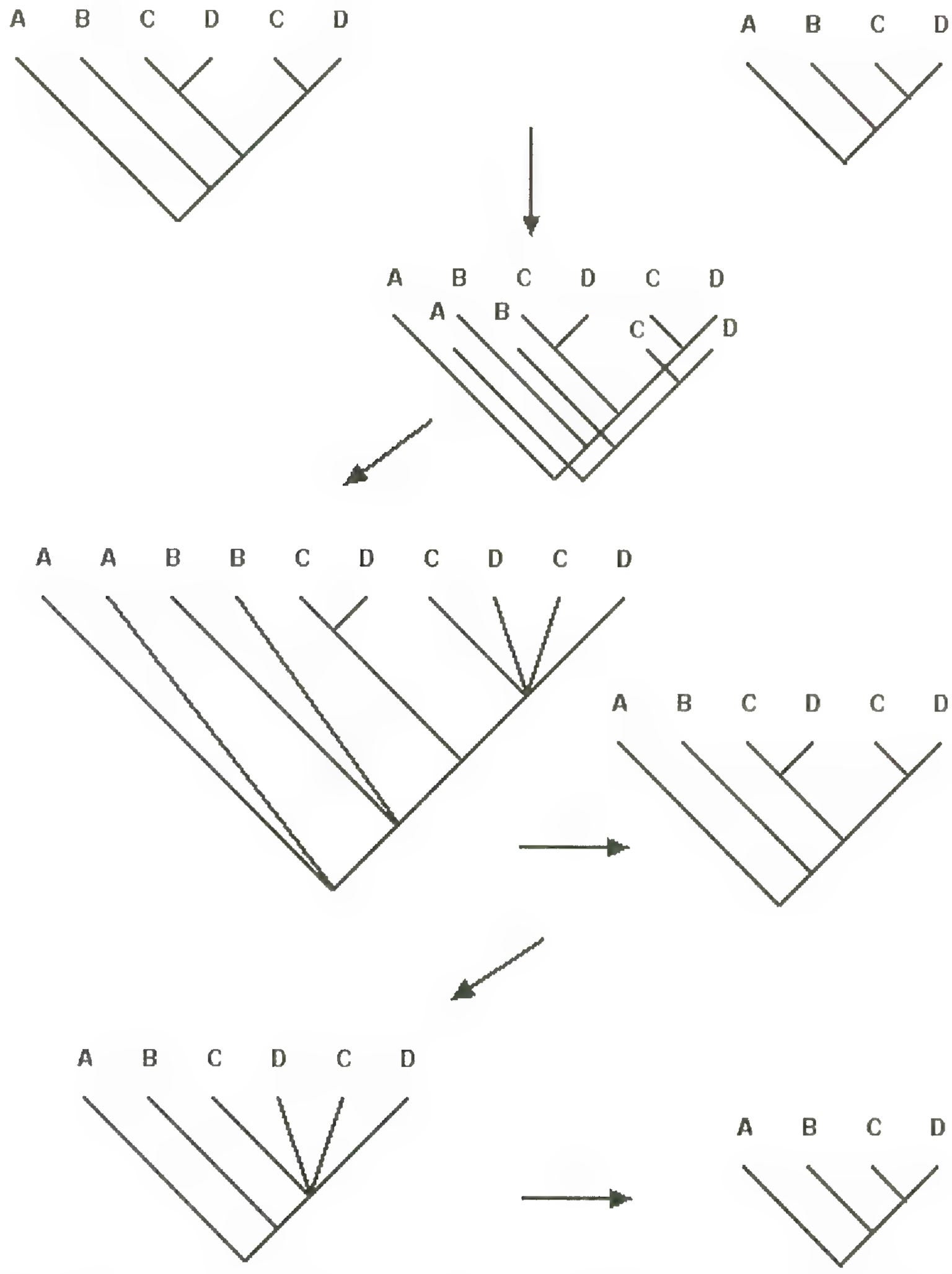


Figure 5. PACT analysis for lineage duplication (sympatric speciation). Top = two hierarchical patterns based on different data but pertaining to entities A, B, C, and D. Second from top = the two hierarchical patterns aligned by common elements. Third from top left = both hierarchical patterns superimposed on each other. Third from top right = PACT solution based on applying combination rule 1 (Y + Y = Y). Note that this leaves duplicate (CD) elements coming from a common node. Bottom left = two (CD) groups coming from a common node superimposed on each other. Bottom right = PACT solution based on applying combination rule 1 (Y + Y = Y).

one infers that data that conflict with the expected pattern (the "law") are the result of historical contingencies, and it is therefore permissible to remove or modify them. As a result, island biogeographers are admonished to study small, young islands in order to minimize the potential for such historical contingencies that cloud our ability to see the true (and simple) pattern.

The second theory of simplicity was the maximum vicariance hypothesis (MaxVic), also known as vicariance biogeography or cladistic biogeography (Humphries & Parenti, 1999). MaxVic is based on the theory that in situ speciation and extinction produce simple area cladograms in which each area occupies a unique position. Noise in the system or the effects of contingency result from dispersal. From this, one infers that data that conflict with a single area cladogram in which each area appears once (the "law") are the result of historical contingencies, and it is therefore permissible to remove or modify them. As a result, cladistic biogeographers developed Assumptions 1 and 2 to remove or modify ("reconcile") incongruent data with a single simple area cladogram in which each area occupies a unique position.

One persistent concern about both these paradigms has been this: if it is necessary to remove and modify data and to restrict one's scope of analysis, just how general and powerful are the explanations produced? A closer comparison of the two paradigms reveals another interesting feature: they are complementary

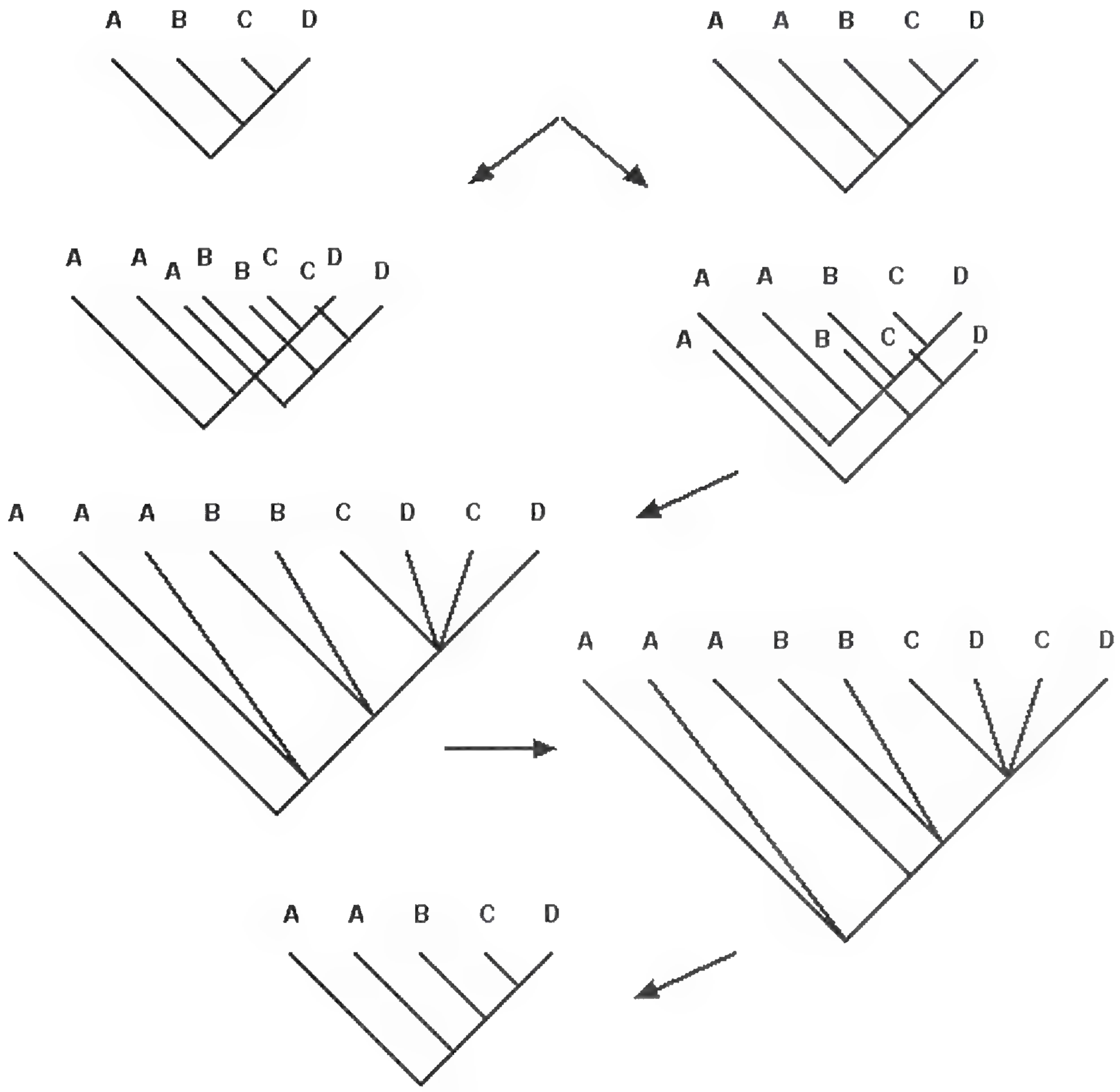


Figure 6. PACT analysis of two partly congruent patterns. Top = two hierarchical patterns based on different data but pertaining to entities A, B, C, and D. Second from top = the two hierarchical patterns aligned by common elements. Third from top = both hierarchical patterns superimposed on each other. Bottom = PACT solution based on applying combination rule 4 (Y(Y– +Y = (Y(Y–. Y (A) (Y– (A– + Y (A–) = Y (A) (Y (A–. This exemplar is also known as the "problem of paralogy."

theories, each one excluding the other's domain of explanation. What is missing from ETIB are assessments of the geographic origin of species, even though this is what distinguishes a "source" from an "island." What is missing in MaxVic are assessments of post-speciation movements, and yet this is how ancestral species become widespread enough to be affected by vicariance. If each of these theories describes something valid and each one excludes the other's explanatory domain, perhaps the problem lies in the adoption of ontological parsimony on the part of advocates of both theories of biogeography.

A "new" guiding principle: The taxon pulse. At nearly the same time the maximum vicariance paradigm emerged, Erwin (1979, 1981) proposed the taxon pulse hypothesis as a model incorporating both dispersal and vicariance. Erwin's model stemmed from an idea proposed by Darlington (1943), later named the "taxon cycle" by Wilson (1959, 1961). Taxon pulse and taxon cycle models both assume that species and their adaptations arise in "centers of diversification," and that distributional ranges of taxa periodically fluctuate around a more stable, continuously occupied center. This general biotic dispersal may be interrupted by the formation of barriers, producing episodes of vicariant speciation. Breakdown of those barriers produces new episodes of biotic expansion, setting the stage for yet more episodes of vicariance. Taxon cycles occur over relatively short periods of time ("ecological time") and involve species that disperse actively and colonize new areas during expansion episodes, then contract their ranges during periods of habitat contraction without producing new species. Taxon pulses, by contrast, occur over relatively long periods of time ("evolutionary time") and are characterized by dispersal along a broad front during expansion into suitable habitat when previous barriers break down. During this expansion phase, different species within

a biota encounter additional geographic heterogeneity, including range contractions. Such heterogeneity may (1) stop the expansion of some species, resulting in species of restricted distributions; (2) affect only the rate of expansion for some species, producing widespread species; or (3) act as barriers to dispersal of sufficient magnitude to produce new species as a result of peripheral isolates speciation. Geological evolution, operating on longer time scales than biological evolution, may also produce barriers, resulting in episodes of vicariant speciation affecting members of these same biotas.

Despite the existence of an alternative to MaxVic and despite concerns that exemplar taxa were being carefully selected to show a preponderance of vicariance (Simberloff et al., 1980; Simberloff, 1987), vicariance has become the default explanation for any observation of allopatry. And yet, MaxVic has always been deficient because it neglects the issue of how ancestral species of many clades become widespread enough to be affected by vicariant events. If vicariance affects many members of ancestral biotas in the same way, it seems reasonable to assume that at some point in the past the members of the biota expanded their geographic ranges to such an extent that they could be affected by the subsequent vicariance event. Advocates of vicariance biogeography have acknowledged that this must happen. Wiley (1981) noted that some circumstances, such as colonization of islands, might produce general distribution patterns based on dispersal rather than vicariance, and Endler (1982) suggested that such correlated dispersal patterns might be common. In practice, however, historical biogeographers have simply assumed that such dispersal does not produce general patterns, so it is permissible to invoke dispersal only to explain departures from the general pattern, which is always explained as the result of vicariance (Wiley, 1986, 1988a, b; Brooks & McLennan, 1991, 2002; Humphries & Parenti, 1999).

Taxon pulse-driven biotic diversification differs from vicariance-driven biotic diversification in three important ways. First, because diversification is driven by biotic expansion, we expect to find general patterns associated with dispersal, not just with vicariance. General patterns resulting from biotic expansion occur when barriers to dispersal, especially the large-scale ones leading to vicariance, break down. Second, episodes of biotic expansion, even those involving large areas, will inevitably lead to reticulated historical relationships among areas and biotas within areas of endemism comprising species of different ages derived from different sources. Third, the absence of particular clades in particular areas is more parsimoniously explained as a lack of participation in that particular expansion episode by a particular clade, rather than dispersal with extinction. Taxon pulses are also historically contingent, meaning that at any given time, different clades comprising a complex biota may form a mosaic of area relationships.

Recent studies have shown extensive reticulated area relationships even for data sets carefully chosen to emphasize vicariance (e.g., Brooks & McLennan, 2001; Green et al., 2002; McLennan & Brooks, 2002; Spironello & Brooks, 2003; Bouchard et al., 2004; Brooks & Ferrao, 2005; Brooks & Folinsbee, 2005; Halas et al., 2005; Folinsbee & Brooks, 2007).

A new research program for historical biogeographic analyses. Following Halas et al. (2005), a new research program based on an ontology of complexity for historical biogeographical analyses comprises two steps:

Step 1—Producing a general area cladogram. Convert all phylogenetic trees into taxon-area cladograms by replacing the names of the species with the areas they inhabit, then combine them using PACT, producing a general area cladogram (GAC). Figure 7 is the GAC resulting from PACT analysis of elephants, hyenas, and hominoids since the Miocene (Brooks & Folinsbee, 2005; Folinsbee & Brooks, 2007).

Step 2—Inferring biogeographic history from the GAC. This is a two-part process. First, we must distinguish general from unique nodes. Unique nodes are produced by an evolutionary event affecting only a single clade. At present, all nodes associated with evolutionary events affecting more than one clade are termed general nodes. Clearly, a general node involving two of 100 clades likely is not the same as one involving 75 of 100 clades. At the moment, however, we do not have enough empirical data or any models to allow us to be more fine-grained in our distinctions among general nodes, but this is clearly an area of interest for future research. Second, we must interpret the general nodes. Until recently, historical biogeographers believed that all general nodes in an area cladogram should be ascribed to vicariance. Lieberman (2000, 2003a, b), however, noted that episodes of geodispersal (geographic areas fusing rather than splitting) could produce general nodes resulting from dispersal. In a similar vein, the taxon pulse hypothesis asserts that episodes of general biotic expansion occurring between vicariance (or, more generally, isolation) events set the stage for future isolation events. Lieberman (2000, 2003a, b) proposed a protocol for distinguishing general nodes in area cladograms due to isolation from those caused by geodispersal or biotic expansion. The protocol is based on phylogenetic character optimization (Fitch optimization), using areas inhabited as the characters.

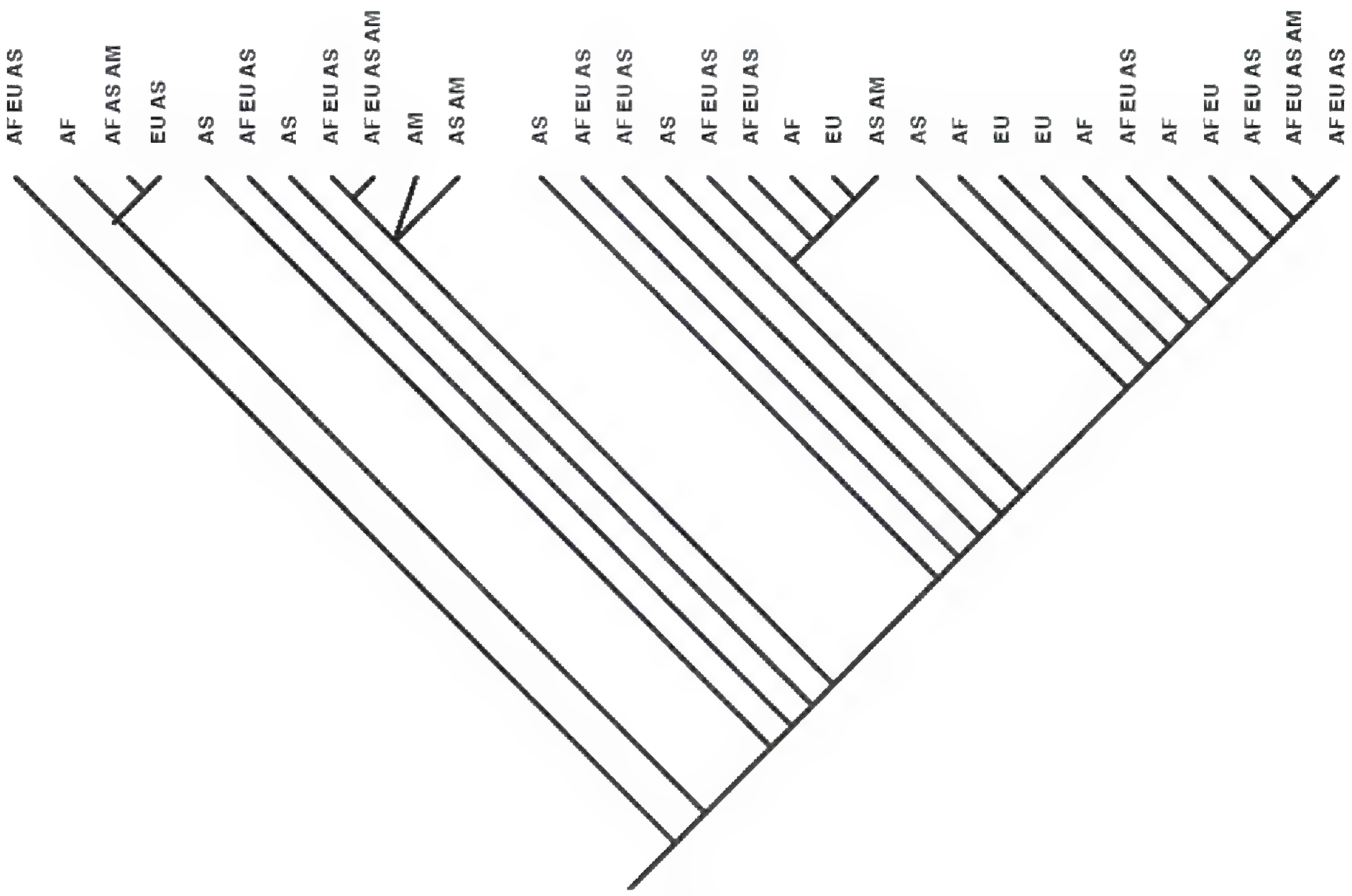


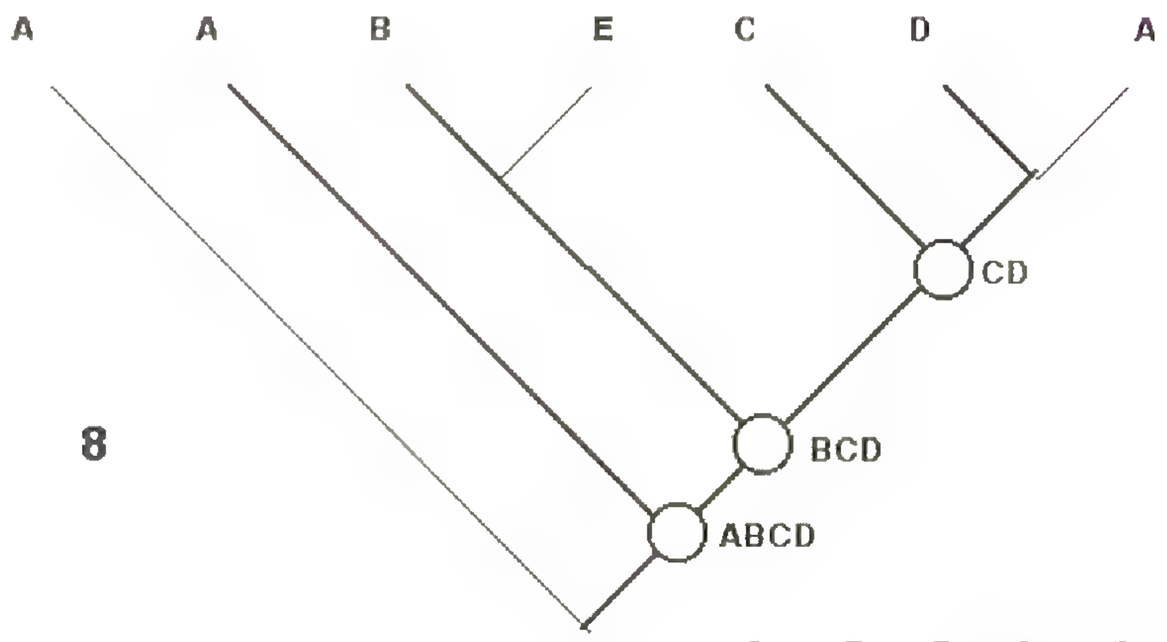
Figure 7. PACT analysis of elephants, hyenas, and hominoids since the Miocene (Brooks & Folinsbee, 2005; Folinsbee & Brooks, 2007). General area cladogram. AF = Africa, AM = Americas, AS = Asia, EU = Europe.

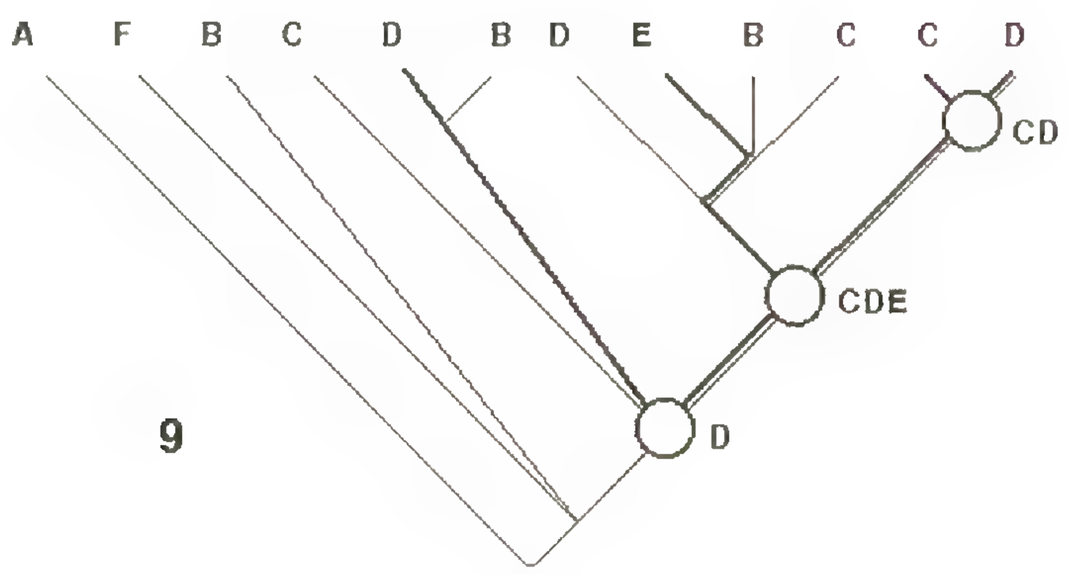
General nodes associated with vicariance or other forms of isolation exhibit decreasing numbers of areas occupied relative to the next oldest node (Fig. 8), whereas general nodes associated with biotic expansion (breakdown of a barrier) are associated with increasing numbers of areas occupied relative to the next oldest node (Fig. 9).

Figure 10 summarizes the nodal analysis for the GAC shown in Figure 7. The alternation of isolation (V, or vicariance) nodes with biotic expansion (BE) and within-area (W) differentiation nodes produces the characteristic signature of a taxon pulse radiation. The relatively large number of general nodes (22 out of 28, or 79%) suggests that all three clades participated in the same general taxon pulse scenario, summarized in Figure 11.

Complexity studies in biogeography: Integrating historical and ecological biogeography. That larger islands have greater species richness than smaller islands, and that "islands" need not be oceanic because species richness increases with any increased sample of area, have long been recognized. ETIB (MacArthur & Wilson, 1963, 1967) predicts a linear log-normal function relationship between species richness and the size of an island resulting from a dynamic balance between immigration, that is, colonization from a source area, and extinction. The extinction rate is assumed to increase with the number of species present on any island, so that small areas with higher species richness than expected have a higher extinction rate than immigration rate and are not yet in equilibrium. MacArthur and Wilson (1963) acknowledged that "local speciation" (in situ speciation) would confound the species-area relationship, but suggested that for most cases it was probably safe to omit in situ speciation from the model as its effect on the species-area relation is "probably significant only in the oldest, largest, and most isolated islands" (1963: 380). Recent discussions, however, have suggested that such historical phenomena require closer investigation (e.g., Heaney, 2000; Whittaker, 2000).

Halas et al. (2005) performed the first direct analysis of the phylogenetic impact on the species-area relationship using the extensive data set presented by Marshall and Liebherr (2000), representing 33 clades of insects, vertebrates, and flowering plants occurring throughout Mexico and parts of Central America. Nodal analysis of their GAC permitted them to classify each species in each area as a colonizer or a resident. Correlating species richness and area size for the 33 clades and nine areas in Mexico and Central America (for details, see Halas et al., 2005) produced a low correlation coefficient for the species-area curve ($r^2 = 0.47$), due primarily to two relatively small areas (the Transmexican Volcanic Belt and the Sierra Madre del Sur) containing unusually large numbers of species. Correlating extinction events and species richness for this data set produced a high correlation coefficient ($r^2 = 0.75$), indicating strong support for this prediction of the





Figures 8 and 9. —8. Lieberman's nodal analysis 1. Thick branches indicate general nodes; thin branches represent elements of the GAC produced by only one clade. Progressive decrease in the number of areas associated with nodal values indicates that all general nodes are isolation nodes. This corresponds to either maximum vicariance or Hennig's progression rule. —9. Lieberman's nodal analysis 2. Thick branches indicate general nodes; thin branches represent elements of the GAC produced by only one clade. Increase in the number of areas associated with nodal values from node D to node CDE indicates an episode of biotic expansion or general dispersal; decrease in the number of areas associated with nodal values from CDE to CD indicates an episode of isolation, corresponding to either vicariance or Hennig's progression rule. Alternation of biotic expansion and isolation nodes is characteristic of taxon pulse radiations.

ETIB. Following Macarthur and Wilson's lead, Losos and Schluter (2000) reported that, for anoline lizards on large Caribbean islands, inferred extinction rates were low and in situ speciation was a more important source of species richness than colonization. They predicted that effects similar to those they observed on the largest islands should be found on continental islands. Halas et al. (2005) corroborated those predictions: in situ speciation correlates better with area size than does colonization, and inferred extinction rates are low. The protocol produces inferences of only 19 extinction events involving 16 of the 33 clades, because direct parsimonious inferences of extinction can be made only for episodes of vicariance. If extinction rates are the same following biotic expansion events, which account for 60% of the general nodes, the number of inferred extinctions increases from 19 to 48, but does not change the correlations with area size.

These data provided additional insight into the evolutionary relationship between colonization and in situ speciation. There is a very poor correlation between colonization and in situ speciation (r^2 = 0.02), indicating that these phenomena are relatively independent of each other. We suggest that the reason historical effects on large islands confound the species-area relationship is the subsequent dispersal of some species produced in situ to other islands, so sources become islands and islands become sources on evolutionary time scales. All nine areas studied by Halas et al. (2005) have served as both sources and islands at different times and to different degrees. Halas et al. (2005) identified six fundamental episodes of vicariance and nine of biotic expansion as the main determinants of the biogeographic patterns observed in the data set. The Transmexican Volcanic Belt has acted as an "island," receiving species by colonization, for all nine biotic expansion events, and the Sierra Madre del Sur has acted as a dispersal source for three of the episodes of biotic expansion. At the same time, both areas were involved in five of the six episodes of vicariance. This explains why these relatively small areas are disproportionately species rich, without any evidence of an accompanying high extinction rate.

The analysis by Halas et al. (2005) indicates that MacArthur and Wilson (1963, 1967) were correct—in situ speciation confounds the species-area relationship. This occurs when the same areas function as islands and as sources at different times. How can this happen? The answer is niche diversification associated with speciation, producing island species that can colonize the source area(s). Niche diversification most often occurs at a much slower rate than

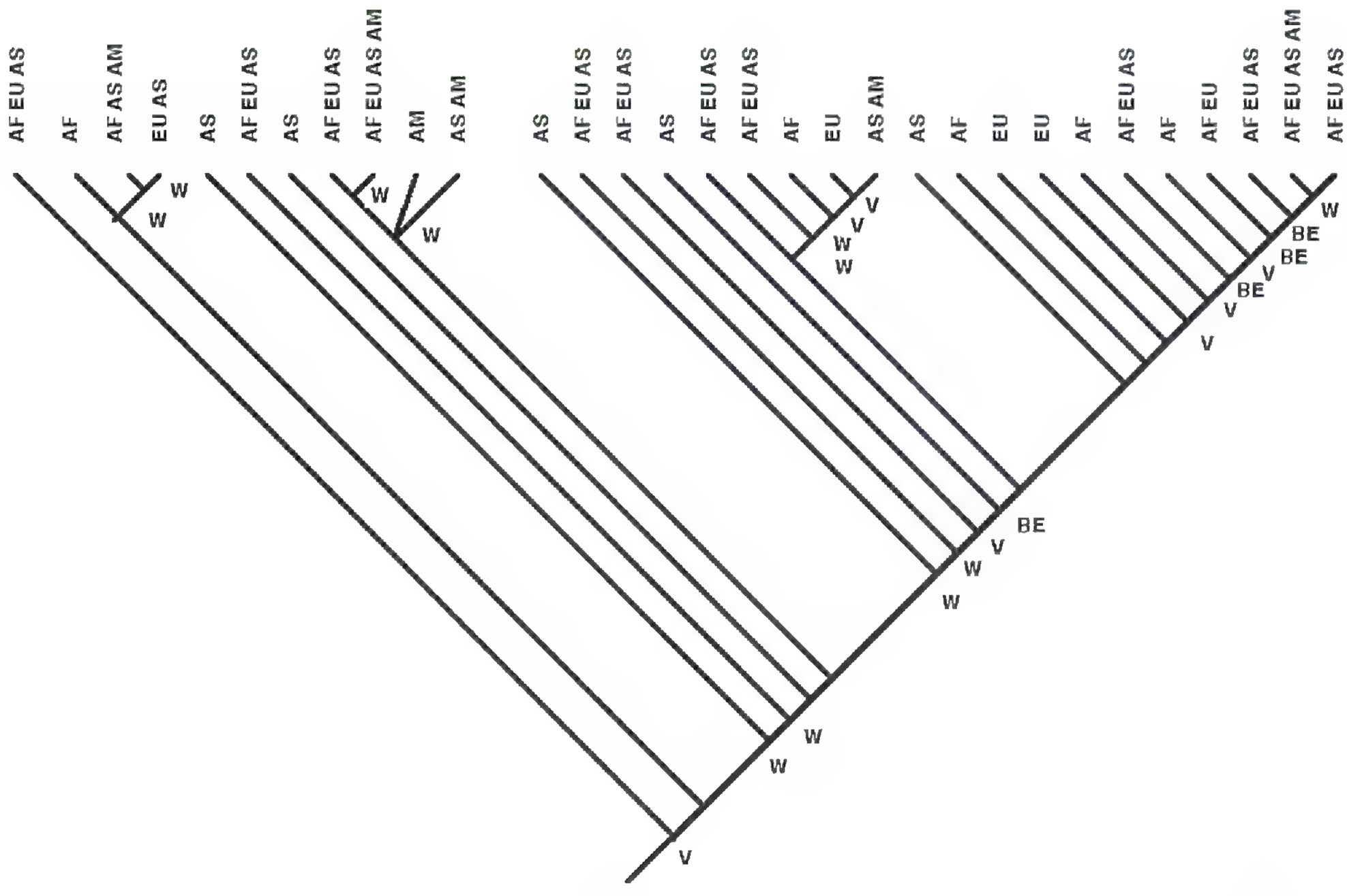


Figure 10. PACT analysis of elephants, hyenas, and hominoids since the Miocene (Brooks & Folinsbee, 2005; Folinsbee & Brooks, 2007). General node analysis. AF = Africa, AM = Americas, AS = Asia, BE = biotic expansion, EU = Europe, V = vicariance, W = within area. Alternation of biotic expansion/within-area and vicariance (isolation) nodes is characteristic of taxon pulse radiations.

speciation (Brooks & McLennan, 2002), but the biogeographic "role reversal" resulting from niche diversification need not occur often in order to affect the equilibrium number of species in a given area. In addition, Cracraft (1985) seems to have been correct in asserting that speciation and extinction are under different rate controls. In the study by Halas et al. (2005), extinction rates correlate well with area size, but speciation rates do not. How can this happen? According to Cracraft (1985), speciation is largely controlled by geography and extinction by environmental harshness, associated with adaptation and niche diversification. That is, extinction is a failure to adapt, not a failure to speciate (Brooks & McLennan, 2002). Occasional episodes of niche diversification plus episodic changes in environmental harshness are the primary source of the "pulses" in the taxon pulse hypothesis. The study by Halas et al. (2005) suggests

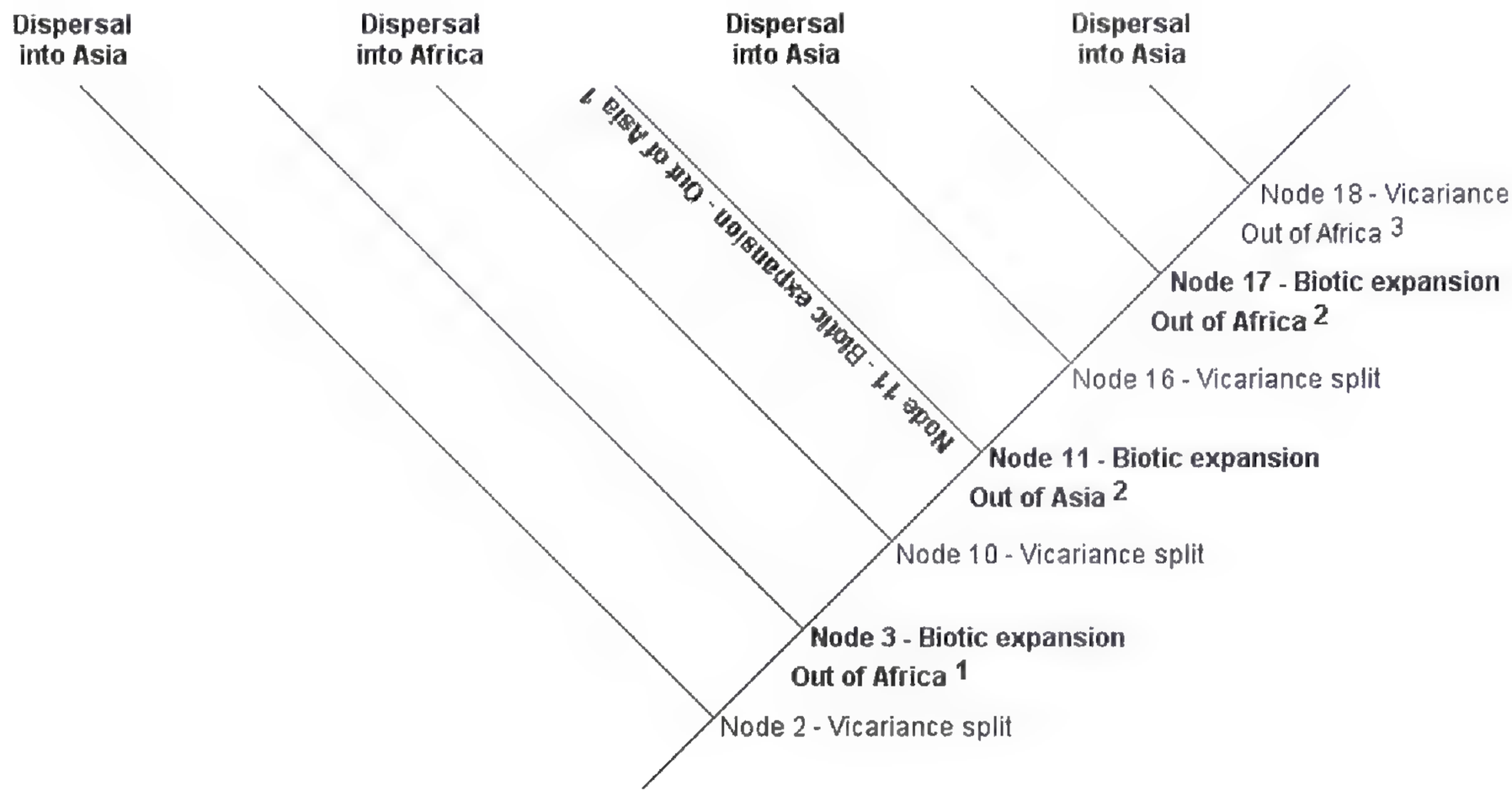


Figure 11. PACT analysis of elephants, hyenas, and hominoids since the Miocene (Brooks & Folinsbee, 2005; Folinsbee & Brooks, 2007). Pictorial representation of the taxon pulse scenario that best explains the data.

that Losos and Schluter (2000) were not correct in one respect: in situ speciation and colonization are not necessarily causally linked, such that high rates of one need not cause low rates in the other. If speciation and extinction are under different rate controls, the appearance of fit to their simple deterministic model was likely the result of using only a single clade for their analysis.

The combination of speciation and niche diversification makes the equilibrium number of species a moving target in evolutionary time; that is, evolution is a non-equilibrium phenomenon (Brooks & Wiley, 1988). This complements recent calls by, e.g., Heaney (2000) and Whittaker (2000) for modifications of the ETIB to incorporate complex patterns of immigration, extinction, and diversification occurring on various spatial scales and on both ecological and evolutionary time scales.

COEVOLUTION

Fahrenholz (1913) focused entirely on the hosts when he postulated that the occurrence of related blood-sucking lice on different primates demonstrated that the catarrhines were more closely related to hominoids than to any other primates. By the late 1930s, this orthogenetic perspective had produced an integrated view of coevolution: parasites are highly host specific, so they coevolve with their hosts, and because they coevolve with their hosts, they become highly host specific. Because host specificity was the *cause* of coevolution rather than a function of the ecological interaction between lineages, any conflicting or inconsistent observations were considered to be erroneous or irrelevant because they failed to conform to the orthogenetic view of coevolution. Vestiges of orthogenetic thinking persist today; this is especially true for the assumption that hosts and parasites "ought" to have congruent phylogenies, embodied in the maximum cospeciation school of research (Page, 2002).

In contrast with the orthogenetic view of coevolution, Kellogg (1896, 1913) cast his observations about host-parasite relationships in a Darwinian framework. His studies of birds and their biting lice suggested that while some host-parasite systems might show strong phylogenetic associations, there were substantial cases of what he termed "straggling" or "host-switching." This perspective was adopted primarily by researchers interested in studying the interactions between plants and phytophagous insects (e.g., Verschaffelt, 1910; Brues, 1920, 1924). Because those associations often showed no clear phylogenetic component with respect to host species (though they often were extremely specific), researchers in this tradition focused on discovering the ecological ties between organisms, particularly the cues insects used to locate their host plants. The modern version of this second perspective on coevolution emerged in the 1960s. Following a mathematical model proposed by Mode (1958), Ehrlich and Raven (1964) hypothesized that the evolutionary diversification of plants and insects had been fueled by complex coevolutionary interactions involving mutual modification. As suggested initially by Kellogg, such coevolutionary dynamics might have a general phylogenetic context, but need not parallel the evolutionary history of the specific taxa involved. The distribution of insects among plants followed the evolution of host resources and the evolution of insects' abilities to utilize those resources, rather than the evolution of host species themselves.

Janzen (1968, 1973a, b, 1980, 1981, 1983, 1985a, b) argued that the appearance of tight coevolutionary associations at any single locality could be misleading. No matter where a given species evolved in the first place, its inherited functional abilities may allow it to survive in a variety of places under a variety of conditions through arbitrary amounts of time. In other words, species and their phylogenetically conservative traits may disperse through time and space. He called this interaction between the past history of the species and their present-day associations "ecological fitting" (Janzen, 1985b).

All perspectives discussed above agree that parasites are resource specialists. They disagree fundamentally on the question of host switching. Can you be a resource specialist and still host switch readily? Brooks and McLennan (2002) discussed a number of ways in which this could happen. Beginning with the parasite, a species might be a resource specialist but also might share that specialist trait with one or more close relatives. That is, specialization on a particular resource can be a plesiomorphic characteristic of a clade of parasites. As a result, a given host species occurring in more than one area might be inhabited by two different species of parasites, related to each other but not necessarily as sister species. Such persistent plesiomorphic traits also might be co-opted to perform novel functions. Trouvé et al. (1998), for example, reported that life history traits of parasitic flatworms do not differ from life history traits of their free-living relatives, indicating that these species do not have a "parasitic mode of life" but rather a "platyhelminth mode of life" that has been co-opted to function in the context of parasitizing vertebrates. Persistent ancestral traits also might be "anachronisms" (Janzen & Martin, 1982), traits that evolved in a coevolutionary context that no longer exists. For example, a number

of members of the Neodermata (the clade of platyhelminths including trematodes, monogeneans, tapeworms, and their relatives) have guts, even though they also have a highly absorptive integument (the neodermis). In the case of the tapeworms, having a neodermis facilitated survival even when the gut was lost. The persistent intestine in other neodermatans is an anachronism, like the vermiform appendix in humans.

Alternatively and from the hosts' perspective, the resources themselves might be very specific and yet still taxonomically and geographically widespread. In such cases, a given parasite might inhabit more than one species of host, the hosts not being sister species, as a result of host switching. Two major categories of parasite specialization are preferred site of infection (Adamson & Caira, 1994; Brooks & McLennan, 1993), as well as their transmission dynamics (Brooks & McLennan, 1993; Hoberg & Adams, 2000; Hoberg et al., 2000).* Phylogenetic conservatism in parasite biology, coupled with phylogenetic conservatism in host biology, would create a very large arena for host switching, even without the evolution of novel capabilities for host utilization.

If historical conservatism in the type of specialization, rather than specialization per se, determines the ease or difficulty of host switching, the extent of such historical conservatism should dictate our expectations about the frequency of occurrence of host switching. Two ubiquitous findings from the historical ecology revolution of the past 20 years are that all aspects of evolution, including ecology and behavior, are phylogenetically conservative, and that host switching is a regular feature of coevolutionary history (Brooks & McLennan, 1991, 1993, 2002). Moving phylogenetic studies of coevolution into the age of complexity thus requires the use of methods of analysis based on the recognition that host switching is common, not rare, and that the fundamental job of the investigator is to explain the switches that have occurred and, where possible, to anticipate switches that could occur rapidly during periods of substantial climate change.

A new guiding principle: The oscillation hypothesis. In comparative studies of coevolution, host shifts, including increasing host range and speciation by host switching, are the analog of dispersal in historical biogeography. Complexity studies of coevolution should, thus, be based on an analog of the taxon pulse hypothesis. Phylogenesis in taxa exhibiting "close and evident" ecological associations with host taxa should be characterized by episodes of increasing host range followed by episodes of isolation on particular hosts. Janz and Nylin (2007) have recently proposed such a model, which they call the "oscillation hypothesis" (Janz et al., 2006; Nylin & Janz, 2007), and provided empirical evidence supporting it based on studies of various lepidopteran taxa and their host plants.

A new research program for coevolution studies. Within the realm of an ontologically complex research program, comparative studies of host-parasite associations use the following two steps:

Step 1—Producing a general host cladogram. Convert all parasite phylogenetic trees into parasite-host cladograms. This is accomplished by replacing the names of the parasite species with the hosts they inhabit. Then combine them using PACT—this produces a general host cladogram (GHC), a map of the host context of parasite speciation. We illustrate this approach using the pinworms (*Enterobius* Leach [1853]) and hookworms (*Oesophagostomum* [*Conoweberia*] Ihle [1922]) inhabiting the great apes including humans (for details with references, see Brooks & McLennan, 2003; Brooks & Ferrao, 2005). The GHC is shown in Figure 12.

Step 2—Assessing the host switches. The heavy branches on the host cladogram (Fig. 12) indicate instances of congruence between host and parasite phylogeny. This includes one case (*Enterobius gregorii* Hugot [1983] and *E. vermicularis* [Linnaeus, 1758] Leach [1853], both inhabiting humans) in which a parasite clade speciated but the host did not, and another (*Oesophagostomum stephanostomum* Stossich, 1904) in which the host clade speciated but the parasite did not. Despite the substantial amount of cospeciation between the parasites, which also happens to be congruent with the host phylogeny, the PACT analysis suggests that about 30% of the observed host associations are due to host switching. Only one represents a switch to a non-primate host (rodents). The remainder, all of which involve primate to primate switches, include acquiring parasites that are more or less the same age (three cases), that are younger (one case), and that are older (three cases) than the hosts.

Complexity studies in coevolution: Integrating biogeography and coevolution. We expect the evolution of increased host range to occur over relatively long time periods (Janz et al., 2006; Janz & Nylin, 2007; Nylin & Janz, 2007). This has led evolutionary biologists to predict that while generalists may be at an evolutionary disadvantage relative to specialists with respect to particular host species in the short term, they are at an advantage in the long term. Generalists have this advantage over specialists because of their ability to utilize multiple hosts, which increases their chances of survival during episodes of major climate change and

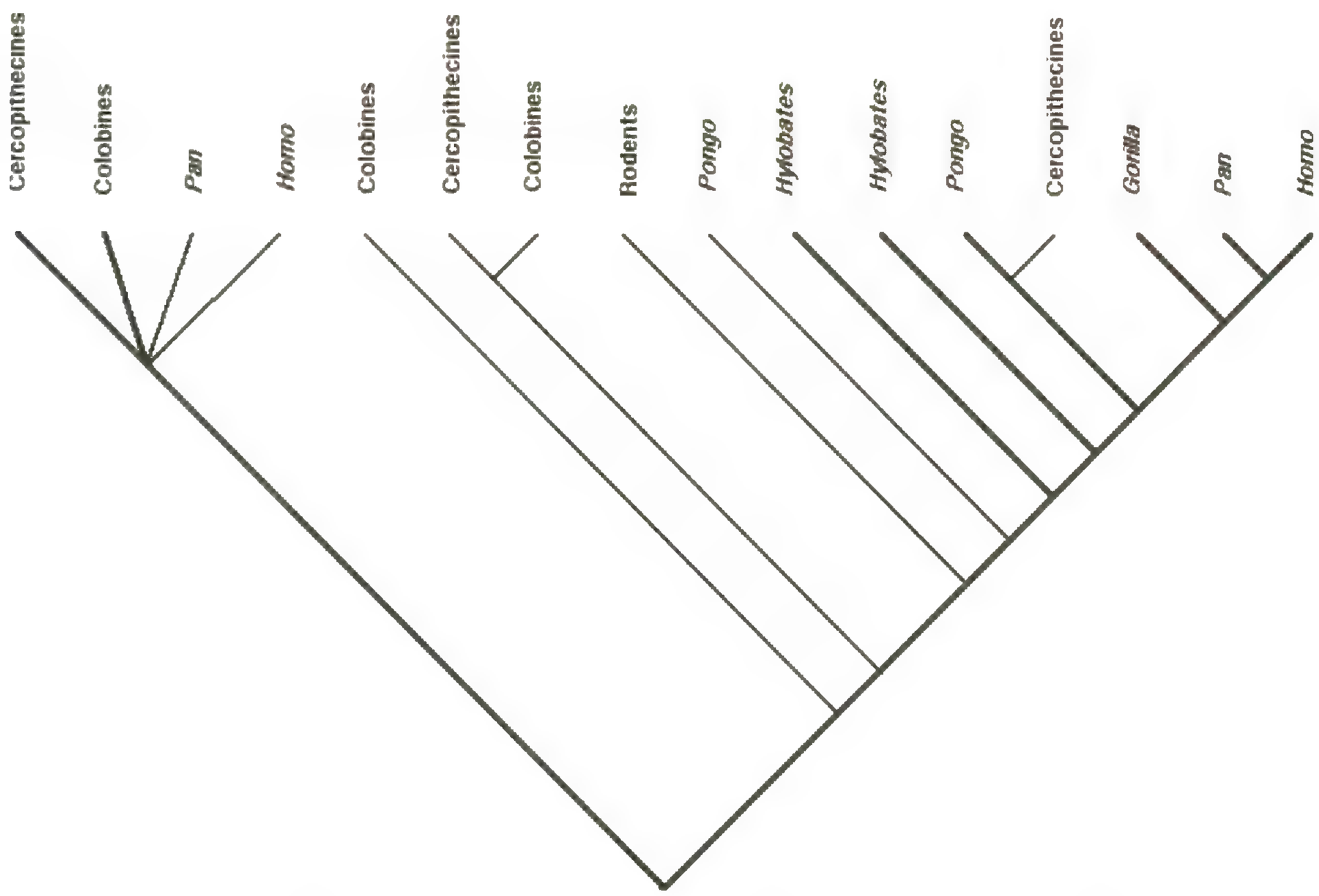


Figure 12. General host cladogram produced by PACT, indicating host context of speciation events implied by phylogenies for *Enterobius* Leach (1853) and *Oesophagostomum* (*Conoweberia*) Ihle (1922). Heavy lines indicate associations congruent with host phylogeny; thin lines indicate host-switching events.

associated changes in species composition and trophic structure in affected ecosystems. Brooks and McLennan (2002) suggested that ecological fitting, in the form of phylogenetically conservative host utilization capabilities, produces a more complex situation. In addition to true generalists, actively using different host species representing different resources, and true specialists, capable of surviving in association with only a single species of hosts, there are what Brooks and McLennan (2002) called "faux generalists" and "faux specialists." Faux generalists are resource specialists whose resource is widespread among host species (synapomorphic, symplesiomorphic, or homoplasious). At any given place and time, only a restricted subset (often only a single species) of all potential host species are available to the specialist, but over a large geographic range, the specialist may be associated with many hosts. By having a large class of potential hosts, this class of specialists may have the same long-term evolutionary advantages as true generalists without giving up their short-term advantages as resource specialists. Faux specialists, by contrast, are resource generalists who, at any given place and time, are excluded from some suitable hosts by specialists on those hosts. By participating in associations with a small number of hosts, such generalists may have many of the same short-term benefits as true specialists, without losing the long-term advantages of being a resource generalist.

The most important aspect of this recognition by Brooks and McLennan (2002) is that true generalists, faux generalists, and faux specialists can all switch hosts rapidly during episodes of climate change. This has significant implications for public, livestock, and wildlife health specialists (Brooks & Hoberg, 2006, 2007a, b). One critical implication of the maximum cospeciation perspective is the expectation that progressive coevolutionary specialization mitigates host switches. This means that coevolution should provide its own safeguards against emerging infectious diseases (EIDs). We would thus expect EIDs to be rare events evolutionarily, and the current crisis would be seen as something different in kind rather than in degree from the evolutionary background of coevolution. Under the Darwinian view, by contrast, EIDs are not rare events but are common evolutionary accidents waiting to happen. In that case, the current EID crisis is different only in degree, not in kind, from coevolutionary dynamics occurring prior to the present. The potential for host switching is therefore proportional to the number of susceptible hosts that are not "apparent" to a parasite at any given time. The apparency arena, although phylogenetically conservative, is not static. Environmental changes in the place of origin for any parasite, leading to altered trophic interactions or to geographic dispersal, increase the chances that the parasite will come into contact with additional susceptible hosts. Each time this happens,

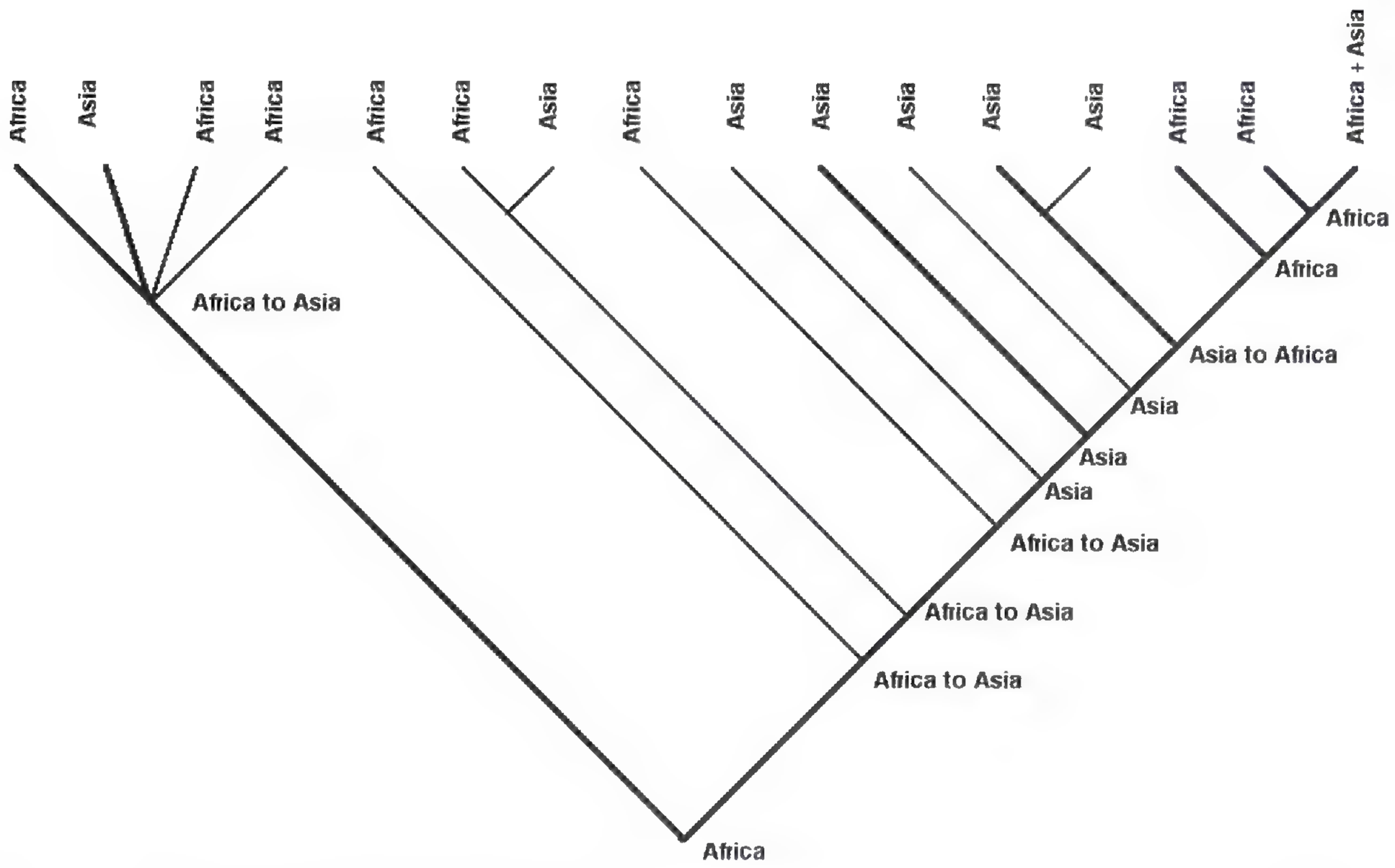


Figure 13. Area cladogram produced by PACT, indicating geographic context of speciation events implied by phylogenies for *Enterobius* and *Oeoshagostomum* (*Conoweberia*). Heavy lines indicate area associations shown by both parasite clades; thin lines indicate area associations shown by only one clade. Areas at nodes indicate episodes of isolation (in either "Asia" or "Africa") or of biotic expansion ("Africa to Asia," "Asia to Africa," or "Asia and Africa").

the parasite becomes an EID of the newly acquired host.

Brooks (1985) first called attention to analytical similarities in phylogenetic studies of coevolution and biogeography. Given this, it is fairly straightforward to integrate information from both types of studies to obtain some understanding of the evolutionary basis of EIDs. When the background is one of alternating episodes of vicariance and biotic expansion, therefore, episodes of rapid host switching associated with the biotic expansion events may result. The biogeographic analysis of *Enterobius* and *Oesophagostomum* (*Conoweberia*) (Fig. 13) suggests a history of speciation involving alternating episodes of isolation in, and then movements between, Africa and Asia. Furthermore, five of the eight (63%) host switches discovered in Figure 12 occurred during periods of biotic expansion discovered in Figure 13. The exceptions are the switch from primates to rodents in the common ancestor of the parasites *O. xeri* Ortlepp (1922) and *O. susannae* Leroux (1940), which took place in Africa, as well as *O. blanchardi* Travassos & Vogelsang (1932) in orangutans and *O. raillieti* Travassos & Vogelsang (1932) in gibbons (perhaps representing a case of sympatric speciation), which took place in Asia. Together, these observations suggest that these host switches, all of which would have been called EIDs at the time of their origins, with the possible exception of the switch from primates to rodent by the common ancestor of *O. xeri* and *O. susannae*, resulted from changes in the apparency arena rather than the evolution of novel capabilities for host-utilization (Brooks & Hoberg, 2006, 2007a, b).

EIDs may also arise from host switches mediated by ecological fitting within the area of origin when climate changes alter trophic structure. For example, when ancestral humans moved out of the African forest and onto the savannah during the late Pliocene and early Pleistocene, they made a rapid transition from herbivory to facultative carnivory to active predation. During that time, humans apparently shared more than just food with other apex carnivores, becoming hosts to species of cestodes in the genus *Taenia* L. (1758), whose closest relatives inhabit hyenas, large cats, and African hunting dogs (Hoberg et al., 2000, 2001). Thus, colonization of hominids was driven by shared trophic structure in foraging guilds where apex carnivores and ancestral humans foraged on an array of antelope prey that served as intermediate hosts for species of *Taenia*.

Until now, PACT has been used to assess biogeographic and coevolutionary associations among species representing multiple clades. Here, for the first time, we demonstrate how PACT can be used to help further studies of species, speciation, and phylogenetics. Because these are new proposals for applications of Assumption 0 analysis, we begin with the discussion of basic principles.

According to Darwin, the origin of species is historically contingent in space and time (Darwin, 1872). Darwinian thought has always considered

species to be complex systems, whether we consider them to be communities of descent (Darwin, 1872), historical lineages (Simpson, 1944; Wiley, 1978, 1981), cladogenetic units (Hennig, 1966), or historically cohesive information systems irreversibly split from other such units (Brooks & Wiley, 1988; Kornet, 1993; Maynard Smith & Szathmary, 1995) through a variety of cohesion-breaking mechanisms (Brooks & McLennan, 2002). Evolutionary phenomena affecting and producing species operate at the level of reproductive and ecological interactions among individual members of various populations that comprise them.

TOKOGENETICS AND PHYLOGEOGRAPHY

One of the most fundamental assertions made by Hennig (1966) was the distinction between "tokogeny" and "phylogeny." He asserted that tokogenetic, or within-species, relationships could not be analyzed using phylogenetic systematic principles due to reticulated breeding relationships among organisms, but that phylogenetic, or among-species, relationships could be analyzed using his method because there were no reticulated relationships in phylogeny. By implication, clades that have experienced episodes of hybrid speciation are excluded from Hennigian analysis. The majority of biologists have accepted this self-imposed limitation until recently (see, e.g., Posada & Crandall, 2001).

A "new" guiding principle. In discussing empirical criteria for demarcation among species, Sites and Marshall (2003, 2004) followed Hennig (1966) and Kornet (1993) in suggesting that populations showing reticulated relationships should be considered a single species, whereas populations not showing reticulated relationships should be considered more than one species. Similarly, Riddle and Hafner (2004) suggested that area reticulations should be more common when dealing with populations of the same species than when dealing with populations of different species. The developments of Assumption 0 and of PACT demonstrate that phylogenetic systematic reasoning can be applied to complex historical systems whose members have reticulated relationships. Therefore, viewed in the light of complexity studies, phylogeography (Avise, 2000) is the study of the history of tokogenetic relationships.

A new research program for tokogenetic analyses. Within the realm of an ontologically complex research program, phylogeographic analyses of entities (populations) that show tokogenetic relationships use the following two steps:

1. PACT analysis of all available data. Convert all gene trees into taxon-area cladograms (Fig. 14). This is accomplished by replacing the names of the haplotypes distinguished by each gene tree with the areas they inhabit. Ideally, both mitochondrial and nuclear genes should be used, the former because they retain patterns of introgression well, and the latter because they provide direct evidence of gene flow. Then combine the taxon-area cladograms using PACT. This produces a GAC (Fig. 15) depicting the geographic context of the deployment of genetic information within and among the geographically defined populations.

2. Assessing species boundaries and patterns of genetic cohesion. As suggested by Sites and Marshall (2003, 2004), the Hennigian distinction between tokogeny and phylogeny provides a straightforward empirical criterion for determining how many different species are represented in a sample of populations. Species (macro-species of Kornet, 1993) are identified by the largest inclusive subset (clade) of populations (micro-species of Kornet, 1993) that show reticulated relationships. Using this formalism, Figure 15 depicts three species, comprising populations a–d, populations e–h, and populations i–l, respectively.

The next step is to ascertain the patterns of genetic cohesion among the populations within each species. A truly panmictic species would be characterized by all populations showing reticulated relationships with each other, producing a GAC in the form of a single unresolved polytomy for all populations. In a Darwinian world, historical contingencies of heredity (the nature of the organism) and circumstances (the nature of the conditions) should produce various degrees of within-species cohesion. The hypothetical three-species system in Figure 15 illustrates this point. Each species is characterized by four populations. Species 1 (populations a–d) has one unique population (d) and three populations exhibiting a total of four reticulations (one each for populations a and c, two for population b), combining for eight inter-population connections. Species 2 (populations e–h) has two unique populations (f and h) and two populations exhibiting a total of two duplications (one each for e and g), combining for 13 inter-population connections. Finally, species 3 (populations i–l) has one unique population (j) and three populations exhibiting a total of four duplications (one each for k and l, two for i), combining for 14 inter-population connections. If the magnitude of gene flow is comparable in all inter-population connections, then species 2 and 3 would be deemed more cohesive than species 1, and species 2 might be considered to have achieved its high degree of cohesion more

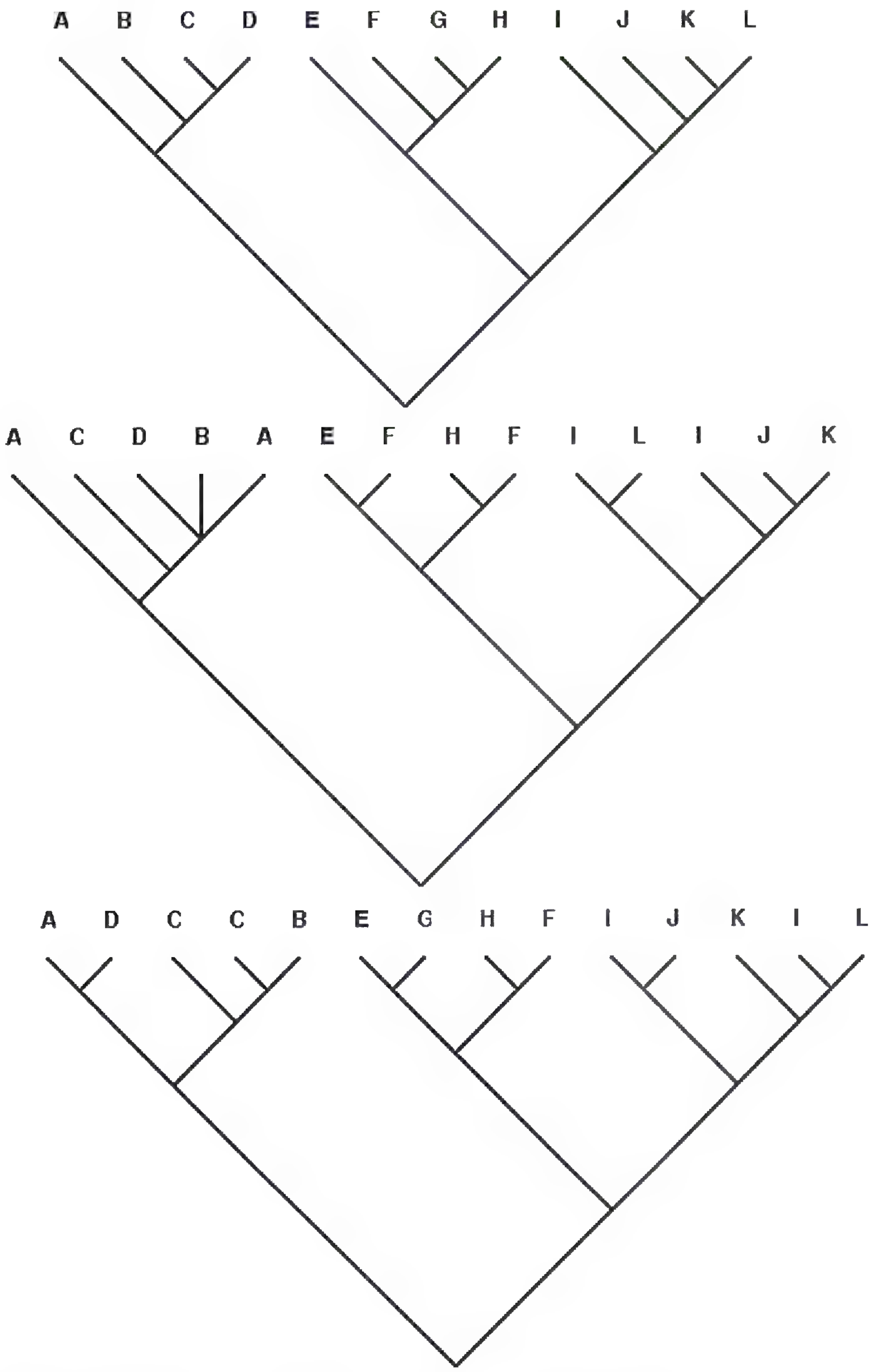


Figure 14. PACT and phylogeography 1. Three gene trees for various populations.

efficiently than species 3, needing only two duplications to achieve 13 inter-population connections as opposed to four duplications to achieve 14 inter-population connections.

PHYLOGENETICS

The Hennigian assumption that phylogeny can be represented accurately by an internested branching diagram is refuted each time a new species is formed by the hybridization of two (or more) other species. This produces a reticulated phylogenetic history that cannot be represented by a purely internested branching diagram that is the result of analyses with standard phylogenetic methods.

Most zoologists consider hybrid speciation to be rare in animals, but botanists have recognized for a long time that many plant species have been formed in this manner (Vriesendorp & Bakker, 2005 and examples therein). Furthermore, the endosymbiont theory of the origin of eukaryotes (e.g., Schwartz & Dayhoff, 1978; Margulis, 1981; Sitte, 1993) postulates that certain major transitions in evolution (sensu Maynard Smith & Szathmary, 1995) are the result of hybridization. At least some prokaryotes have an interesting ability that refers to the capability of

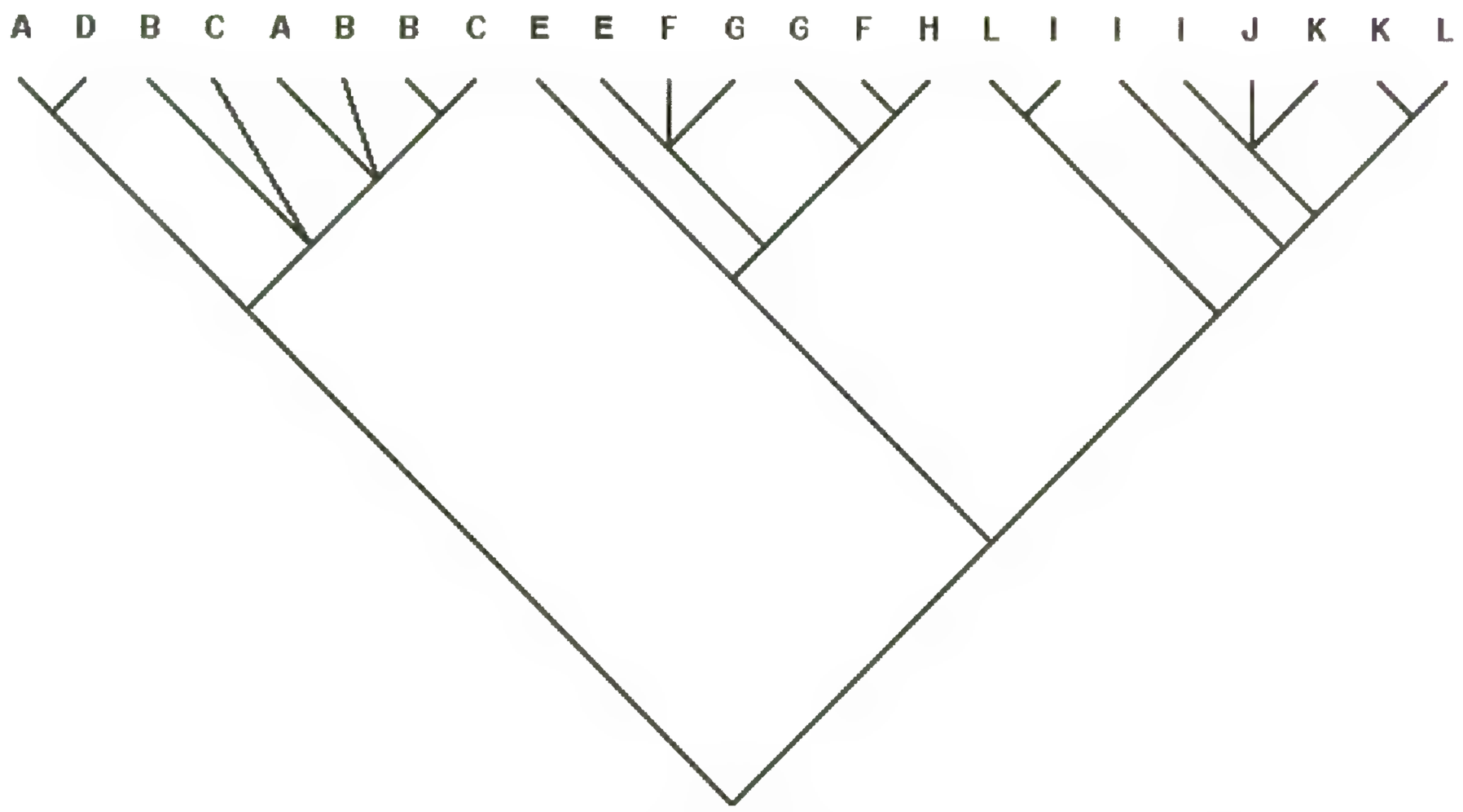


Figure 15. PACT and phylogeography 2. PACT analysis of three gene trees shown in Figure 14.

producing reticulated phylogenetic relationships. In times of stress, these prokaryotes not only shut down their metabolic activities but may actually jettison the portion of their genomes associated with metabolic activities. Furthermore, they are capable of regaining genetic machinery for metabolic activities, but not always from a close relative.

Partial hybridization is a common process for prokaryotes (Woese, 2000, 2002). In bacteria, genes for resistance to antibiotics are usually situated on plasmids. These plasmids behave as accessory bacterial chromosomes and can be transferred by bacterial conjugation. Their plasmids can be exchanged via a tubular connection between two bacterial cells, and resistance to antibiotics can become widespread to relative as well as nonrelative species. Another way of transferring genetic material between two species of bacteria can be accomplished by transduction by a bacterial virus (bacteriophages or phage). This partial hybridization by exchange of genetic material, however, is not only restricted to bacteria. In case of bacterial transformation (by, e.g., *Agrobacterium tumefaciens* [Smith & Townsend, 1907] Conn [1942] or *A. rhizogenes* [Riker et al., 1930] Conn [1942]), genetic material may even be transferred from a prokaryote to a higher plant species.

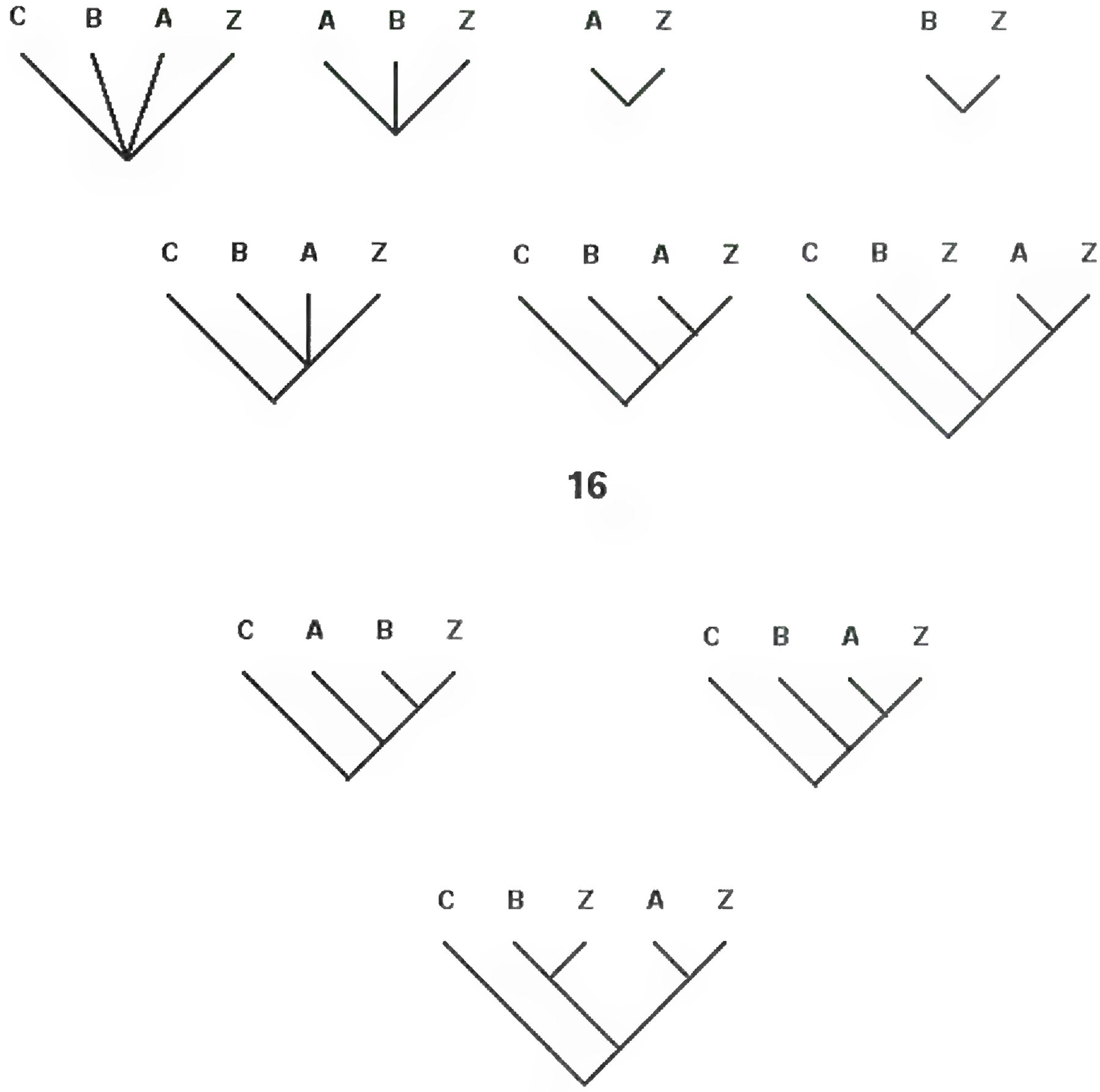
A "new" guiding principle. Phylogeneticists were aware early on that species of hybrid origin might pose special problems (Bremer & Wanntorp, 1979; Funk, 1981), and this was the subject of a symposium at the second meeting of the Willi Hennig Society (Humphries, 1983; Nelson, 1983; Wagner, 1983; Wanntorp, 1983). Funk (1985), however, was the first to propose that analyzing correlated homoplasies in analyses of clades containing species of hybrid origin could be used to detect the hybrid species. She noted that when there are multiple equally parsimonious trees for a clade suspected of containing species of hybrid origins, those trees often represent a small number of distinct topologies. Furthermore, inspection of the trees indicated that most of the homoplasy and, thus, most of the ambiguity was contributed by a small number of species. Removal of those species from the analysis produced a stable topology and reduced homoplasy. Funk's insights, combined with PACT, form the basis for an approach to phylogenetic analysis under Assumption 0.

A new research program for phylogenetic analyses. Within the realm of an ontologically complex research program, phylogenetic analyses of entities show cladogenetic as well as reticulated relationships, and the following two steps are applied.

Step 1—PACT analysis of all available data. Convert all transformation series into character-state trees, then combine them using PACT (Figs. 16, 17), ignoring the plesiomorphic state. This produces a phylogenetic tree in which all traits interpreted as homoplasy by standard Hennigian analysis will be interpreted as homologies resulting from horizontal transfer/hybridization.

Step 2—Explaining reticulations. There are two different, yet intertwined, issues involved in this step. The first is differentiating reticulated homology from true homoplasy. The second is differentiating horizontal transfer from hybrid speciation.

The key to deciding the first issue is having phylogenies based on as many characters and character types as possible—comprehensive total-evidence studies. We would then expect, as suggested by Funk (1985), that cases of hybrid speciation would



Figures 16 and 17. —16. PACT and analysis of hybrid species. Top four are binary character-state trees indicating distribution of apomorphic states. Bottom three are PACT results of (from left) combining 1 and 2, then (1 + 2) and 3, then (1 + 2 + 3) and 4. —17. PACT and analysis of hybrid species. Top 2 diagrams are gene trees; bottom diagram is PACT result for combining the gene trees. Parental species are sister species.

appear in a PACT analysis as duplications supported by many characters (because hybrid speciation involves combinations of whole genomes), whereas cases of true homoplasy would appear as duplications supported by single characters (because homoplasy involves single characters). For such cases, relying on epistemological parsimony would have an ontological justification. Consider this thought experiment:

One species (Z) forms a sister relationship with each of two other species (A and B) in a PACT analysis. If you have one case of (BZ) and one of (AZ), there are three possible explanations: (1) hybrid speciation (Assumption 0 default); (2) one case of horizontal transfer (A is the true sister species of Z, with horizontal transfer to B or vice versa); or (3) one case of true homoplasy (independent origin). Using epistemological parsimony alone, there is no way to choose, based on the evidence at hand.

Next, suppose you have five cases of (BZ) and five cases of (AZ). Now the explanations become (1) one case of hybrid speciation; (2) five cases of horizontal transfer or one to four cases of horizontal transfer of linked traits; and (3) five cases of independent evolution or one to four cases of "co-adapted trait complex" or "adaptive syndrome" evolution. In this case, epistemological parsimony would lead us to choose hybrid speciation unambiguously as the preferred explanation. That means we hypothesize that all shared similarities among the species are homologous.

This approach is not guaranteed to produce correct results, of course. It works when enough of the heritage of each parental species is expressed in the hybrid product(s). We expect that the more distantly related the parents are, the less likely we will be able to recover the parental species, but also the less likely

that hybrid speciation will occur. In addition, we often expect hybrid species to segregate phylogenetically more with one parent than another (see McDade, 1990, 1992; Mindell, 1992, 1993; Zrzavy & Skala, 1993; Skala & Zrzavy, 1994). For cases in which ambiguity in phylogenetic analysis results from a small number of species and when there is abundant evidence of correlated homoplasy, Funk's approach gives us an excellent first approximation as to the possibility that the ambiguity is due to those species being hybrids, justifying the use of Assumption 0 analysis. However, when larger numbers of species are involved, the explanations for character ambiguity may become very complex.

For example, recent studies of prokaryote phylogenetics have raised the possibility that this propensity on the part of prokaryotes has produced some wildly reticulated phylogenetic relationships. Some have even expressed the opinion that this means we should change our view of phylogeny, from a tree to a reticulated network (Doolittle, 2000). Maynard Smith and Szathmary (1995), however, pointed out that genetic machinery associated with storing and transmitting information shows no similar evidence of horizontal transfer. Thus, the true phylogeny may still be tree-like in pattern, although substantial homoplasy in metabolic genes may be present due to horizontal transfer.

Regardless of the eventual outcome of this debate, we do not have to develop fundamentally different approaches to assessing the evidence about relationships. It simply means that the more horizontal transfer there has been, the more we will have to resort to species duplications to find the source of those transfers. Much more work, including substantial modeling effort, is needed to help determine when it is appropriate to use Assumption 0 in phylogenetic analysis and when it is not.

We will now address the second issue. When Assumption 0 and PACT are applied in this context, all reticulations are taken as evidence of introgression, but introgression can produce multiple outcomes, of which speciation is only one. Differentiating the various forms and outcomes of introgression requires a robust and unified ontological species concept (the evolutionary species concept; Simpson, 1944, 1951; Wiley, 1978) as well as a broad-based epistemological view of the diversity of kinds of species. As we suggested earlier, the PACT analysis is the beginning of an explanation, not an explanation in itself. Inference of species boundaries requires additional tests (such as nested clade analysis) to highlight the independent lineages and experiments to determine the strength of cohesion of those lineages (for a discussion and protocol, see Brooks & McLennan, 2002).

Finally, we come to the issue of how to incorporate reticulated phylogenetic events into our classifications. There are two choices. Do we classify a tree with reticulations, or do we try to classify a reticulated network? If we choose the former, hybrid species will appear in two different clades, reflecting their phylogenetic origins. This disturbs traditional classifications in which each taxon has a unique place. If we choose to try to classify a network, each species of hybrid origin would be classified separately from either parental species, so the resulting classification would maintain the tradition of each taxon having a unique placement, but could not provide information about the phylogenetic relationships of the hybrid species. If we wish our classifications to reflect what we think we know about evolution, it seems that we will have to opt for the first alternative.

Conclusions: Back to the Future

Evolution is historically contingent and complex. Darwinism provides an ontology of complexity. Historical contingency requires phylogenetic information to document and explain patterns. Representing this complexity so that we have some understanding of what needs to be explained requires a method that allows reticulated relationships to be found. A new algorithm, PACT (Wojcicki & Brooks, 2004, 2005), has been developed for that purpose. Applying this algorithm in historical biogeographical, coevolutionary, phylogeographical, and phylogenetic studies invokes Assumption 0: all data must be analyzed without modification. Thereby, the resulting patterns of related areas, species, and populations contain duplications of entities to such an extent that these patterns are logically consistent with all input data. Further inference of these duplications asks for an ontology of complexity in which multiple evolutionary processes resulting in both divergent and reticulated patterns nest comfortably.

Methods for phylogenetic analysis that only deal with divergent patterns and do not allow duplication of entities disallow complex evolutionary processes for explaining the convergent (reticulated) patterns and thereby oversimplify the historical relationships among the entities of analysis. Furthermore, several studies mentioned in this paper show that several older and newer concepts like the taxon pulse (Erwin, 1979), ecological fitting (Janzen, 1985b), phylogenetic analysis of hybrids (Funk, 1985), and the oscillation hypothesis (Janz & Nylin, 2007) allow for explanation of reticulated patterns.

In order to perform comparative analyses based upon an ontology of complexity, we do not have to begin again because Darwinism is based on such an ontology. We do, however, have to move forward,

beyond the oversimplification of evolution associated with much of 20th-century evolutionary biology. Basic principles of phylogenetic systematics, coupled with Assumption 0, can accommodate analyses of complex evolutionary associations at various levels of organization. Once we begin doing this, we will recapture earlier ideas and integrate them into a renewed appreciation for the Darwinian panorama.

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RESOLVING SPECIES PHYLOGENIES OF RECENT EVOLUTIONARY RADIATIONS[1]

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ABSTRACT

For recently derived species and when the time separating speciation events is short, the phylogenetic distribution of taxa in a gene tree may not accurately reflect the actual species relationships. The phylogenetic tradition of relying on gene tree and species tree synonymy is not reliable under such historical scenarios. Nevertheless, recent studies have demonstrated that accurate estimates of species relationships are possible when the method of phylogenetic inference considers not only the stochastic processes of nucleotide substitution, but also the random loss of gene lineages by genetic drift—even when there is widespread incomplete lineage sorting. This simulation study examines how the broader phylogenetic context, that is, the species tree topology and branch lengths, influences the ability to recover species relationships when taxa have undergone a recent and rapid radiation. As expected, the time since species divergence and the time between speciation events influences whether phylogenetic relationships are accurately estimated. However, the influence of the timing of divergence on the ability to recover species relationships accurately differed depending on the relative position of the taxa in the species tree. Differences in the ability to recover these relationships across multiple simulated species trees highlight the potential effects of taxon sampling on phylogenetic inference at, or near, the species boundary and under high rates of speciation. By focusing attention on the species tree, rather than on the individual gene trees as the basis for interpretations about species relationships, these results also represent a fundamental shift from the phylogenetic paradigm.

Key words: Coalescence, divergence time, genealogical discord, incomplete lineage sorting, radiation, speciation, statistical phylogeography.

Within a traditional molecular phylogenetic framework, species relationships are inferred directly from the topology of the estimated gene trees (Felsenstein, 2004). Under this paradigm, phylogenetic resolution (at the most basic level) becomes a question of how many unlinked loci are necessary to provide corroborative evidence of species relationships (e.g., Miyamoto & Fitch, 1995; Poe & Chubb, 2004; Jennings & Edwards, 2005; Rieseberg et al., 2006) or number of nucleotide sites required to estimate a gene tree (or topology from combined data across multiple loci) (e.g., Walsh et al., 1999; Rokas et al., 2003). However, when the time separating species divergence is short, genetic drift is unlikely to have time to bring loci to fixation before subsequent speciation events (Tateno et al., 1982; Tajima, 1983; Pamilo & Nei, 1988). Consequently, the estimated gene tree has limited utility because the most recent common ancestor of individuals is not likely to occur within a species lineage.

The problems that incomplete lineage sorting (i.e., the failure of gene lineages to coalesce into a common ancestor before subsequent species divergence) poses for estimating species relationships are widely recognized—the genealogical histories of individual loci may not faithfully reflect the phylogenetic history because of retention and sorting of ancestral polymorphism (Avise et al., 1983; Pamilo & Nei, 1988; Takahata, 1989; Maddison, 1997; Rosenberg, 2002, 2003). To avoid misleading conclusions about species relationships, arising from either discord between a gene tree and the species tree (Maddison, 1997) or widespread incomplete lineage sorting that obscures any obvious pattern of historical relatedness (e.g., Jennings & Edwards, 2005; Maddison & Knowles, 2006; Knowles & Carstens, 2007a), methods of phylogenetic inference need to take into account the process of gene-lineage sorting (e.g., Maddison & Knowles, 2006; Liu & Pearl, 2007). These methods shift the focus away from the idiosyncrasies of individual gene trees as the basis for inferring phylogenetic relationships, to estimating the history of species divergence (i.e., the species tree) directly (e.g., Carstens & Knowles, 2007; Edwards et al., 2007).

One of the conditions where the stochastic loss of gene lineages by genetic drift and incomplete gene-lineage sorting will make gene trees an unreliable basis for inferring taxonomic relationships involves

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the rapid diversification of species. During evolutionary radiations (e.g., Shedlock et al., 2004; Nikaido et al., 2006), speciation events occur in rapid succession. Consequently, reconstructed gene trees may not accurately reflect the history of speciation among taxa because of the persistence of ancestral polymorphism across subsequent species divergence events. In fact, the expected lack of congruence among independent genes (Hasegawa et al., 1985; Slowinski, 2001) has been used to infer that species have radiated (e.g., Jackman et al., 1999; Poe & Chubb, 2004).

Rather than commenting on the discordant genealogical histories as evidence for a radiation, here we examine whether a signal of species relationships can be extracted despite the genealogical discord that accompanies rapid speciation. We consider the effects of both the timing of species divergence and the internode distance (i.e., the timing of the preceding speciation event) on recovering phylogenetic relationships when there is widespread incomplete lineage sorting. The ability to estimate the phylogenetic relationship of a pair of sister taxa is explored over a natural range of branch lengths and topologies, rather than focusing on a specific species tree (e.g., Takahata, 1989; Rosenberg, 2002), thereby providing a broad historical context for examining how the history of species divergence during a recent radiation will affect our ability to recover species relationships.

Methods

Procedural Rationale

The information for estimating phylogenetic relationships is extracted from the pattern of gene-lineage coalescence (as described in Maddison & Knowles, 2006), as opposed to synonymizing the gene trees with the species tree. We focus on historical scenarios where the hazards of incomplete lineage sorting are expected to predominate—namely, recently diverged species. Because the probability that a particular gene tree for a population model can be calculated under the coalescent process (Takahata & Nei, 1985; Rosenberg, 2003; Degnan & Salter, 2005), a species or population tree can be inferred (in principle) using a full-probabilistic model (e.g., Maddison, 1997; Degnan & Salter, 2005). In practice, Maddison and Knowles (2006) showed that the species history can be accurately inferred, even if the actual probabilities of incomplete lineage sorting are not quantified under a stochastic model. Therefore, an approach that incorporates the genetic process that results in the coalescence of gene lineages below the divergence of the species (i.e., deep coalescence) during the phylogenetic inference procedure is applied here.

Computer Simulations

Species trees that include a natural spectrum of topologies and branch lengths were simulated using Mesquite version 1.1 (Maddison & Maddison, 2004). Species trees were simulated for six species to have a total time depth (summed length of branches from any terminal down to the root) of 100,000 generations according to a Yule model. With an N_e of 100,000, a total time depth of 100,000 generations (i.e., a total tree depth of 1 N) leads to considerable incomplete lineage sorting (e.g., Fig. 1). One thousand species trees were simulated, 100 of which were selected at random as species trees in which a particular taxa pair was sister (species E and F were randomly chosen as the target sister taxa), out of the 109 species trees with E and F as each other's closest relative, and species A was the outgroup. To account for the stochasticity of the genetic process of gene lineage coalescence, the ability to recover the sister relationship of species E and F was examined over 100 replicate data sets for each species tree, in which either one locus or three loci were sampled for each of the 10 individuals in each species. Gene genealogies for each replicate were simulated by a neutral coalescence (Kingman, 1982; Hudson, 1990) through Mesquite's Neutral Coalescence module, which uses an exponential approximation to avoid fully explicit modeling of individuals. A biologically reasonable effective population size (N_e) of 100,000 was used for all simulations. Population size of the ancestral species lineage was also set to the common size of 100,000; this represents a reasonable null model of speciation. Otherwise, varying the effective population size of the ancestor would imply some sort of demographic event (e.g., a bottleneck or expansion if its size was set to smaller or larger values, respectively, compared to the descendent taxa).

A species tree was then estimated for each replicate from the simulated gene trees by minimizing the number of deep coalescences between the gene trees and the species tree (for details, see Maddison & Knowles, 2006). This method is based on searching for the species trees that minimize the implied number of deep coalescences in the contained gene trees (Maddison, 1997). The number of deep coalescences was counted assuming the reconstructed gene trees were unrooted and using an "as is" taxon addition sequence, followed by subtree pruning regrafting branch swapping, saving only a single tree at any stage (MAXTREES = 1).

Statistical Analyses and Summary of Results

To evaluate the ability to recover the sister relationship of E and F, each replicate was examined

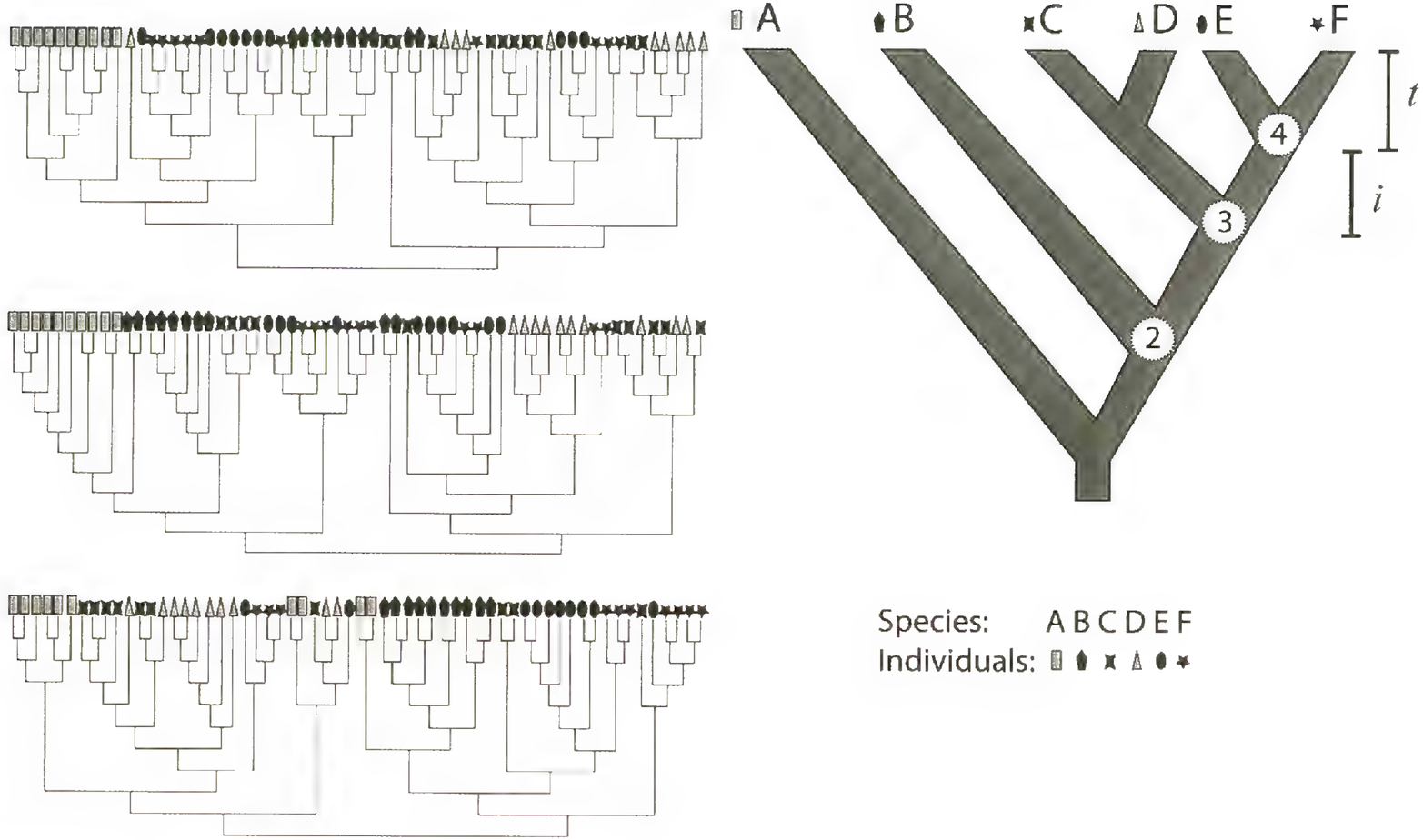

Figure 1. Example of the degree of incomplete lineage sorting apparent in a gene tree of any single sampled locus and the degree of discord among sampled loci for the recent radiation of species studied here (on the left are three representative gene trees); the effects of the species divergence time (t), internode distance (i), and nodal position (shown in the white circles) of the sister taxa relative to the other species on recovering species relationships accurately are considered here. Gene trees were simulated with 10 gene copies (i.e., individuals) per species by neutral coalescence within a simulated species tree (shown on the right) with a total depth of species tree from root to tips = 1 N, where N = 100,000.

and the number of times species E and F were estimated as sister taxa was recorded. To examine what factors influence the accuracy of estimated relationships, (1) the depth of the species divergence, (2) the time of the preceding speciation event (i.e., the length of the internode defining the sister taxa E and F), as well as (3) the position of the E and F species split relative to the other four taxa in the original species tree were noted (Fig. 1).

Analyses of variance (ANOVAs) were used to test for a relationship between divergence time and internode distance (Fig. 1) on the ability to recover the sister relationships of species E and F. An analysis of covariance (ANCOVA) was used to test whether this relationship differed depending on the relative position of the target taxa in the species tree (i.e., nodal position). To separate the effect of nodal position from the influence of differences in the timing of divergence on the accurate recovery of E and F as sister taxa, this analysis was carried out on the residuals from the regression of phylogenetic recovery and divergence time.

Results and Discussion

For the shallow species histories considered here, there is a very low probability of reciprocal monophyly of the species (Hudson & Coyne, 2002), and incomplete lineage sorting and gene tree discord predominate (see also Maddison & Knowles, 2006; Knowles & Carstens, 2007b). With a total tree depth of 1 N_e, which corresponds to 100,000 years assuming one generation per year and an effective population size of 100,000 for each species, the timing of species divergence and interval between speciation events is very short (see Fig. 1). For example, the average depth of the divergence of species E and F ranged from 0.01 to 0.16 N_e and averaged 0.1 N_e, or approximately 10,200 years assuming one generation per year, whereas the average internode distance, or the time of the preceding speciation event, ranged from 0.002 to 0.03 N_e with an average of about 2300 years (Fig. 2). The rate of speciation in the simulations (i.e., six species originating with the total tree depth of 100,000 years) corresponds to the origin of a new species about every 17,000 years. Therefore, the simulations serve as a guide to the conditions affecting phylogenetic accuracy when species have undergone an evolutionary radiation (e.g., Mendelson & Shaw, 2005). The range of species divergence times represented in the simulation study is also common to species-level studies (Arbogast et al., 2002), especially those involving Pleistocene divergences (e.g., Knowles, 2000, 2001; Masta & Maddison, 2002; Hewitt, 2004), making the study broadly relevant to the general difficulties with reconstructing species relationships at or near the species boundaries.

While the discordance between gene trees and species trees (Edwards & Beerli, 2000; Knowles & Maddison, 2002; Hey & Machado, 2003) makes interpreting the genealogical patterns observed in any single gene tree problematic (see Fig. 1), as shown by Maddison and Knowles (2006), the phylogenetic relationships of species can nevertheless be accurately inferred despite widespread incomplete lineage sorting. However, study design is critical to such inferences (Takahata, 1989; Rosenberg, 2002). This study shows that the accuracy of phylogenetic estimates differed depending on the number of loci considered, where accuracy reflects the proportion of replicates in which species relationships were estimated correctly for each species tree. A higher percentage of correct species relationship was recovered with three loci compared to one locus (average of 59.4 $\pm$ 1.81 standard error vs. 48.2 $\pm$ 1.37 standard error, respectively).

Both species divergence time and the internode distance explained a significant amount of the variance in phylogenetic accuracy across the species trees (Fig. 3). However, internode distance had a much stronger influence on phylogenetic accuracy compared to species divergence time. The ability to estimate the sister relationships of species E and F (e.g., Fig. 2) decreases dramatically with a short time interval between the divergence time of species E and F and the preceding speciation event (i.e., small internode distances). This matches theoretical expectations that demonstrate that not only the timing of species divergence, but also the interval between speciation events, strongly influences the consistency probability between species and gene trees (Takahata, 1989; Rosenberg, 2002). Even with very recent species divergence (i.e., less than 0.1 N_e), the relatedness of species E and F was recovered accurately using genealogical information from a single locus (Fig. 2). However, if the timing of the E and F species divergence was similar to that of the preceding speciation event (i.e., short internode distance), there is a low probability of recovering the sister relationships of E and F, even with three loci (Fig. 3). ANCOVA showed that when controlling for differences in divergence time (Table 1), internode length still explained a significant amount of the variance in phylogenetic accuracy across the different species trees when both a single locus and three loci were used to estimate the species tree ($r^2 = 0.80$, $P < 0.0001$, and $r^2 = 0.68$, $P < 0.0001$, for the whole model for one locus and three loci, respectively).

The timing of divergence is not the only factor that affects phylogenetic accuracy—the relative position of taxa in the species tree (i.e., nodal position) also

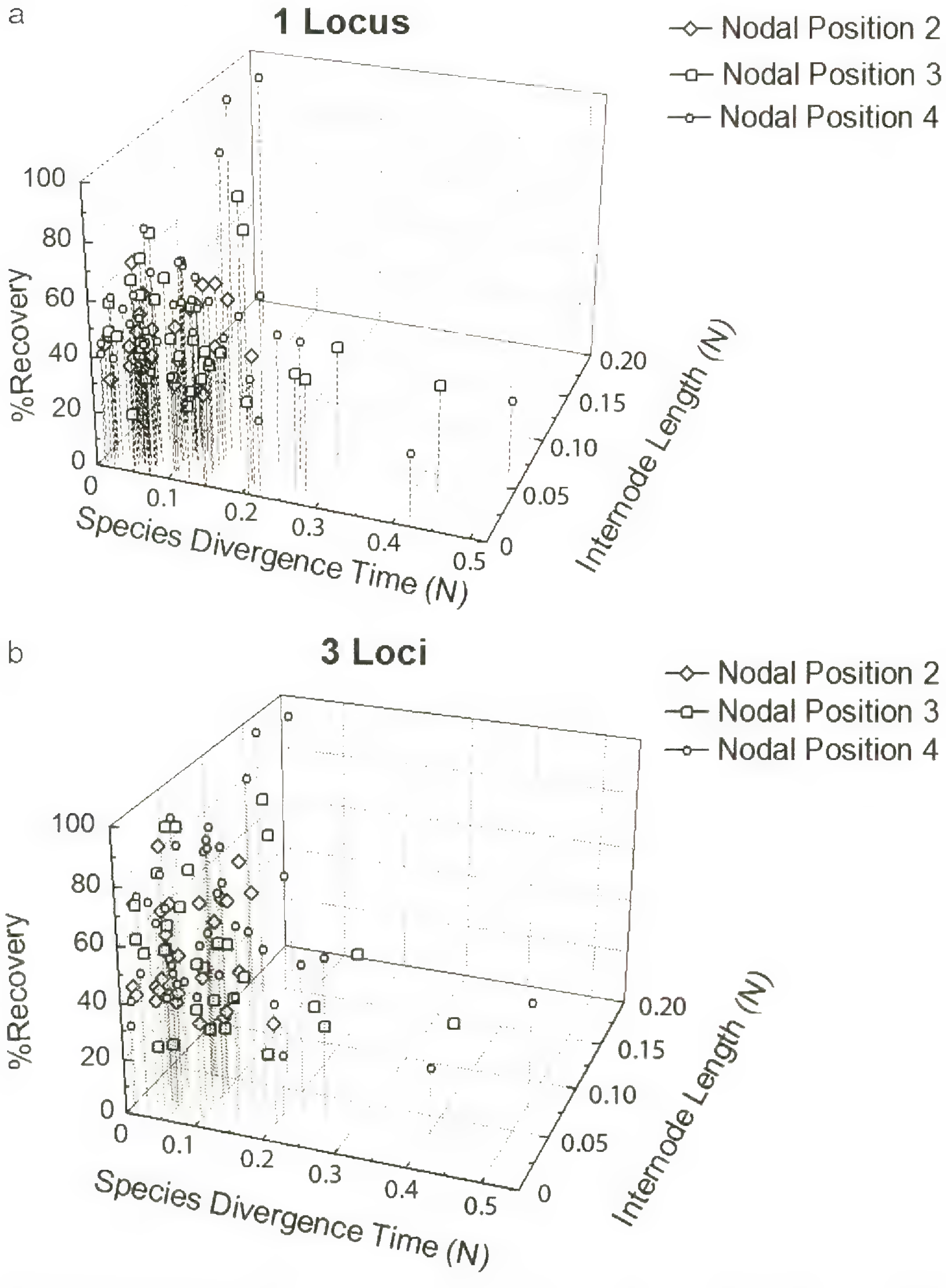

Figure 2. Phylogenetic accuracy across the range of species divergence times and internode lengths represented in the 100 simulated species trees when species relationships are estimated from one and three loci, respectively; the position of the focal taxa relative to the other species (i.e., nodal position) is marked by the different shapes.

impacted whether the sister relationships of E and F were accurately recovered (Table 1). Moreover, nodal position also influenced the effect of internode distance on phylogenetic accuracy (i.e., the interaction term in Table 1). These effects on the ability to reconstruct the sister status of E and F no doubt reflect the probability that gene lineages from species E and F are likely to reach as deep as two, three, or more of the preceding species branchings (see also Shedlock et al., 2004). In addition to the obvious implication this observation has for the effect of species sampling on phylogenetic accuracy, it also has

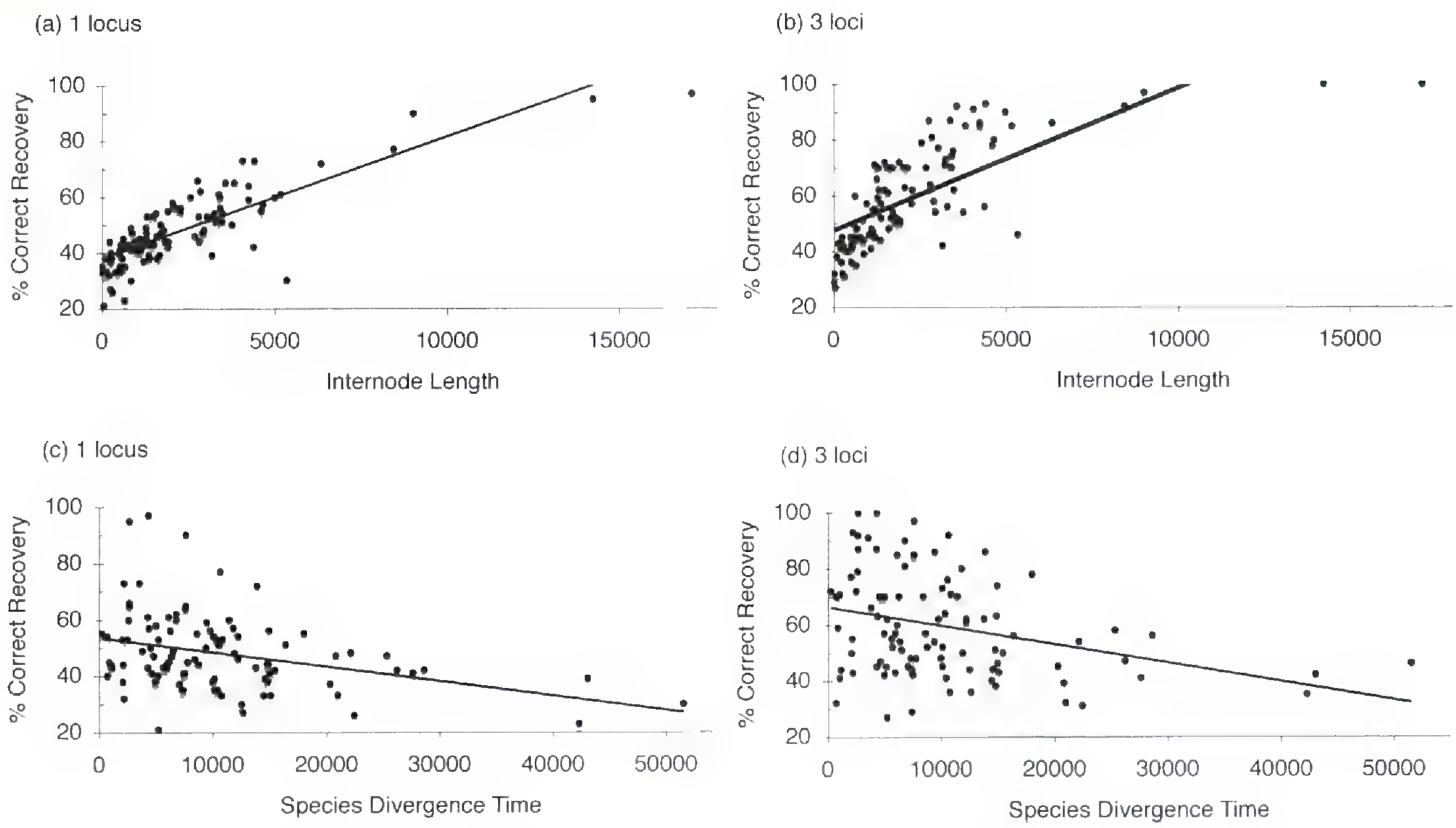

Figure 3. Phylogenetic accuracy (percent of the 100 replicate data sets for each of the 100 species trees in which the correct species relationships were recovered) is significantly influenced by both the length of the internode leading to the target taxa ($r^2 = 0.80$, $P < 0.0001$ for one locus [a] and $r^2 = 0.68$, $P < 0.0001$ for three loci [b]) and the species divergence time ($r^2 = 0.36$, $P < 0.0007$ for one locus [c] and $r^2 = 0.24$, $P < 0.001$ for three loci [d]); the effect of divergence time and internode length has been isolated (i.e., ANCOVA based on either the residuals from a regression of percent correct recovery on either internode length, or divergence time, with node position as a covariate).

important consequences for the ability to recover species relationships during evolutionary radiations. Because the species tree influences the distribution of gene genealogies (see also Degnan & Salter, 2005), the ability to recover the sister relationship of a particular pair of taxa depends on their placement relative to other species, in this case, the placement of species E and F relative to the other four species (Fig. 1). Further investigation of this effect will provide important clues into how the ability to reconstruct these relationships will change over time with the sorting of ancestral polymorphism.

The ability to recover the species relationships is unfortunately expected to diminish with the sorting of ancestral polymorphism when using an approach such as minimizing the number of deep coalescents, as suggested by the decrease in accuracy with increasing species divergence time (Fig. 3), since any signal apparent in the pattern of incomplete lineage sorting will be lost with time. While this suggests limited application of this approach for inferring relationships during evolutionary radiations that have occurred in the more distant past, it could be used as a tool to evaluate whether or not such relationships are likely

Table 1. Phylogenetic accuracy increases with increasing internode length (where, to control for differences in divergence times, the analyses were based on the residuals from a regression of accurate phylogenetic recovery on the timing of species divergence). However, this relationship depends on the shape of the species tree (i.e., the relative position of the target taxa in the species tree) as is evident from the significant effect of node position and significant interaction term in the ANCOVA.

	Degrees of freedom	Sum of squares	F-ratio	Prob > F
One locus				
Internode distance	1	7168.88	207.30	< 0.0001
Node position	2	311.52	4.50	0.0136
Internode distance × node position	2	576.41	8.33	0.0005
Three loci				
Internode distance	1	13369.94	136.38	< 0.0001
Node position	2	1512.06	7.71	0.0008
Internode distance × node position	2	2388.90	12.18	< 0.0001

to be estimated accurately from DNA sequences. This general issue of which particular evolutionary scenarios will defy accurate phylogenetic estimation has been largely overlooked despite its obvious implications for historical inference (see also Knowles & Maddison, 2002).

Conclusion

Despite the relatively modest sampling (i.e., one or three loci sequenced in 10 individuals per species) and challenging conditions (i.e., the radiation of six species over a time spanning just 1 N generations) considered here, species relationships were nonetheless accurately estimated for many of the individual species histories examined (Fig. 2). As with estimates of population genetic parameters (Felsenstein, 2006), increased sampling of loci will no doubt also increase the accuracy of estimated species relationships when the process of gene lineage coalescence is incorporated into the phylogenetic approach (Liu & Pearl, 2005; Maddison & Knowles, 2006; Carstens & Knowles, 2007). Moreover, these findings have important implications for how taxon sampling may influence the ability to recover species relationships and point to further investigation into how phylogenetic accuracy may shift as the time since speciation increases and when taxa have undergone a rapid radiation.

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LIFE HISTORY PATTERNS AND BIOGEOGRAPHY: AN INTERPRETATION OF DIADROMY IN FISHES[1]

Lynne R. Parenti[2]

ABSTRACT

Diadromy, broadly defined here as the regular movement between freshwater and marine habitats at some time during their lives, characterizes numerous fish and invertebrate taxa. Explanations for the evolution of diadromy have focused on ecological requirements of individual taxa, rarely reflecting a comparative, phylogenetic component. When incorporated into phylogenetic studies, center of origin hypotheses have been used to infer dispersal routes. The occurrence and distribution of diadromy throughout fish (aquatic non-tetrapod vertebrate) phylogeny are used here to interpret the evolution of this life history pattern and demonstrate the relationship between life history and ecology in cladistic biogeography. Cladistic biogeography has been mischaracterized as rejecting ecology. On the contrary, cladistic biogeography has been explicit in interpreting ecology or life history patterns within the broader framework of phylogenetic patterns. Today, in inferred ancient life history patterns, such as diadromy, we see remnants of previously broader distribution patterns, such as antitropicality or bipolarity, that spanned both marine and freshwater habitats. Biogeographic regions that span ocean basins and incorporate ocean margins better explain the relationship among diadromy, its evolution, and its distribution than do biogeographic regions centered on continents.

Key words: Antitropical distributions, biogeography, diadromy, eels, fishes, global biogeographic regions, life history, migration.

> *"The extremely slow growth of the larvae of the European eel is...an adaptation to the prolonged journey [to spawning grounds in the Sargasso Sea]. It is scarcely possible to understand this unique phase in the life cycle of the European eel on the hypothesis that the geographical conditions were formerly the same as now exist. But if Wegener's theory [of continental drift] be accepted, the explanation is simple. As the coasts slowly receded from one another the larval life of what became the European species was more and more prolonged by natural selection in correspondence with the greater distance to be traversed."*
>
> —Fulton (1923: 360)

Animals that migrate between marine and freshwater at some time during their life are diadromous, a term coined by American ichthyologist George S. Myers (1949a). Several categories of diadromy were defined or redefined (Myers, 1949a, b; McDowall, 1988)—anadromy, catadromy, and amphidromy—collectively referred to as diadromy herein. Some invertebrate taxa, such as atyid crustaceans and neritid gastropods, are also diadromous (e.g., Myers et al., 2000).

Thomas Wemyss Fulton (1855–1929) was a Scottish fisheries biologist best remembered today for his classic *The Sovereignty of the Sea* (Fulton, 1911), a treatise on the development of the notion of territorial waters. Fulton's life began in 1855, a few years before Charles Darwin's publication of *On the Origin of Species* in 1859, and ended in 1929, just as Alfred L. Wegener published the fourth edition of *Die Entstehung der Kontinente und Ozeane*, the proposal of a theory of continental drift. In his letter to *Nature*, quoted above, Fulton described a hypothesis for the evolution of catadromy in the European eel, *Anguilla anguilla* (L.), which incorporated modern concepts of panbiogeography. First, the earth and its biota evolved together, a tenet of botanist Léon Croizat (1958). Second, global biogeographic regions should be centered on ocean basins, not continents (Croizat, 1958; Craw et al., 1999: figs. 6–13). Third, following from the first two, migration patterns evolve in concert with geology, and these changes lead to lineage differentiation, here changes in length of larval period, and ultimately to what we recognize as

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speciation. When Fulton referred to "... what became the European species...," he postulated that there had been a widespread northern Atlantic lineage that, along with geological evolution of the Atlantic Ocean, differentiated to become the European eel in the eastern North Atlantic and the American eel, *A. rostrata* (Lesueur), in the western North Atlantic. *Anguilla anguilla* and *A. rostrata* are hypothesized sister lineages (Lin et al., 2001a, 2005; see below).

Other early 20th-century biologists adopted earth history to explain the evolution of migration patterns. Notable among ornithologists was Albert Wolfson of Northwestern University, Illinois, who, in 1948, wrote a lead article in *Science*, "Bird Migration and the Concept of Continental Drift," which correlated the increasing distances birds traveled with the separation of land masses and their latitudinal shift. Nearly four decades later, Wolfson (1986) vividly recounted the negative and positive reaction to his correlation of bird migration and continental drift.

Many modern explanations for the evolution of migration and diadromy conflict with those of Fulton and Wolfson because, for the most part, they reject the coupled evolution of life and earth, at global and local scales, and rely exclusively on ecological and evolutionary models. For example "...the relative productivity or growth advantage of sea and freshwater habitats appears to be the key to [diadromy's] evolution. The productivity differential can probably explain why fish migrate across the sea–freshwater boundary, predict their direction of movement and account for where in the world diadromous species occur" (Gross, 1987: 21). Earth history was separated from biological history, in part as a return to center of origin explanations for geographic distributions as outlined by Matthew (1915) in his influential, often reprinted "Climate and Evolution" (see Nelson & Ladiges, 2001). Equally influential was Myers, who classified fishes in ecological categories for the purposes of biogeography. A decade before he defined diadromy, Myers (1938) proposed divisions of freshwater fish taxa based on their ability to tolerate saltwater: freshwater fishes, including primary division freshwater fishes (no salt tolerance), secondary division freshwater fishes (some salt tolerance), and peripheral or migratory fishes (diadromous) versus marine fishes (salt tolerant). These categories have been incorporated into fish biogeography and are still used broadly today (e.g., McDowall, 1988; Helfman et al., 1997; Berra, 2001). (The category of primary division freshwater fishes was modified by paleontologist Colin Patterson [1975], who recognized archaeolimnic taxa, those inferred to have originated and always lived in freshwaters, and telolimnic, those living in freshwater now, but not necessarily throughout their history. These two categories have been used almost exclusively by paleoichthyologists [e.g., Hilton, 2003].) Following Myers's ecological classification, the distribution of primary division freshwater fishes has been interpreted with respect to earth history (viz. Darlington, 1957), whereas the distribution of secondary division freshwater fishes, peripheral fishes, and marine fishes has largely not (e.g., Briggs, 1974, 1995; but see Springer, 1982; Parenti, 1991; Mooi & Gill, 2002; Nelson, 2004).

Accepting Myers's ecological categories precludes the comparison of distribution patterns among diadromous, freshwater, and marine fishes: "It is plainly evident that a fish which can swim through sea water from one river mouth to another is not of much use in studies of terrestrial zoogeography" (Myers, 1938: 343). And so it was believed by Myers and contemporaries (e.g., Herre, 1940) and subsequent generations of biogeographers. Adhering to Myers's philosophy, biogeography of marine and freshwater fishes on a global scale remains largely independent. Textbooks and courses have traditionally drawn the line, contrasting *Zoogeography of the Sea* (Ekman, 1953) and *Marine Zoogeography* (Briggs, 1974) with *Zoogeography of Fresh Waters* (Bănărescu, 1990, 1992, 1995), for example. More general texts, such as *Zoogeography: The Geographical Distribution of Animals* (Darlington, 1957), *Global Biogeography* (Briggs, 1995), or *Biogeography, an Ecological and Evolutionary Approach* (Cox & Moore, 2005), likewise have divided their attention between marine and freshwater taxa.

The arguments for dispersal (e.g., Gross, 1987; McDowall, 1970, 1988, 2001, 2002; Berra et al., 1996; Keith, 2003), vicariance (e.g., Rosen, 1974; Croizat et al., 1974), or some combination of the two (e.g., Choudhury & Dick, 1998) as explanations for the distribution of diadromous taxa, especially the antitropical salmoniform fishes, have been debated extensively. Likewise, broad geographic ranges have been interpreted as explicit evidence of dispersal (e.g., McDowall, 2001, 2002), of vicariance (e.g., Rosen, 1974), or of either dispersal or vicariance (Leis, 1984). Fulton's explanation for *Anguilla* Schrank distribution (above) will not convince a dispersalist to embrace vicariance or a vicariance biogeographer to adopt dispersalist explanations, as each represents a particular view of the relationship between the organism and the environment in forming biogeographic distributions.

Cladistic biogeography (viz. Nelson & Platnick, 1981; Humphries & Parenti, 1986, 1999; Crisci et al., 2003) puts primary emphasis on the phylogenetic relationships among organisms, not their physiological or ecological requirements, to discover distribution

patterns. Cladistic biogeographers do not ignore ecology (viz. Wiens & Donoghue, 2004), but, rather, are explicit in interpreting ecology or life history patterns within the broader framework of phylogenetic patterns (e.g., Sparks & Smith, 2005). Detailed reviews and commentaries on the systematics, biology, and distribution of diadromous fishes, largely within the dispersalist framework, have been provided by New Zealand ichthyologist Robert M. McDowall (e.g., 1970, 1987, 1988, 1992, 1993, 1997a, b, 2001, 2002, 2003). I have argued previously that the distribution of each genus of diadromous gobies of the subfamily Sicydiinae (family Gobiidae) may be described by the ocean basins in which it lives and, further, allopatry may be recognized at the generic level: the eastern Pacific and Atlantic *Sicydium* Valenciennes does not overlap with its sister genus *Sicyopterus* Gill in the Indian Ocean and western and central Pacific (Parenti, 1991; Parenti & Thomas, 1998).

My purpose here is to reinterpret the phylogeny and global distribution of fishes, both freshwater and marine, within a cladistic biogeographic framework to ask if, and if so, how, the biology and ecology of diadromous fishes are related to their distribution patterns and to competing models of earth history.

Diadromy and Classification of Fishes

Table 1 lists the major groups of living aquatic vertebrates that we call fishes, the estimated number of species in each taxon, the number of species that live almost exclusively in freshwater, and the total number of species that enter freshwater during some phase of their lives (modified from Nelson, 2006: 4–5). Diadromous species are included in the last column, which also enumerates those species that do not undergo regular habitat migrations but are euryhaline, such as cyprinodontiform killifishes (see, e.g., Rosen, 1973). An estimated 225 species are diadromous (McDowall, 1992; Nelson, 2006). Not all diadromous species are obligately so (McDowall, 2001), precluding a precise enumeration of such taxa.

A large proportion of diadromous species is concentrated among inferred basal lineages: lampreys (Petromyzontiformes), sturgeons (Acipenseriformes), eels (Elopomorpha), herring (Clupeomorpha), and salmon (Protacanthopterygii), as noted by McDowall (1988, 1993) and others, and many species in these lineages are euryhaline. Many, but not all, diadromous fishes are also antitropical or bipolar, distributed in the northern boreal and/or the southern austral zone, and absent from the tropics, again as noted by McDowall (1988, 1993) and others. Salmoniforms and osmeriforms (Protacanthopterygii; Rosen, 1974: fig. 15), lampreys (Petromyzontiformes; Berra, 2001: 6, 8, 12) and some clupeomorphs (Nelson, 1986) have bipolar distributions, whereas sturgeons (Acipenseridae; Choudhury & Dick, 1998; Berra, 2001: 42) and sticklebacks (Gasterosteidae; Berra, 2001: 351) are boreal, for example.

Epicontinental Seas: The Setting for Bony Fish Evolution

Living actinopterygian (ray-finned) fish clades are the bichirs (Polypteriformes), sturgeons and paddlefishes (Acipenseriformes), gars (Lepisosteiformes), bowfins (Amiiformes), and the bony fishes (Teleostei; see Nelson, 2006). Relationships among these lineages have been debated by both morphologists and molecular biologists (e.g., Nelson, 1969a; Patterson, 1973; Wiley & Schultze, 1984; Inoue et al., 2003) and, for the purposes of this discussion, may be summarized by the cladogram of Figure 1.

Polypteriformes (e.g., Britz, 2004) live in African freshwaters. Fossil bichirs of Africa and South America are of Middle Cretaceous age (Nelson, 2006).

Acipenseriformes (Grande & Bemis, 1991) comprise the anadromous and freshwater circumboreal sturgeons, Acipenseridae, and the paddlefishes, Polyodontidae. There are two extant species of paddlefish, one in freshwater in North America and the other in freshwater and estuarine habitats in the Yangtze River and associated areas of China. Fossil paddlefishes date to the Lower Cretaceous from China (Berra, 2001). Sturgeons are an old lineage, known from at least the Middle Jurassic (175 million years ago [Ma]; Choudhury & Dick, 1998).

Lepisosteiformes includes one living family, Lepisosteidae (Wiley, 1976), which comprises two extant genera with seven species living in fresh, brackish, or marine waters in North and Central America and Cuba. Fossil gars are more broadly distributed throughout North and South America, Europe, Africa, and India (Wiley, 1976: fig. 69); they date from at least the Early Cretaceous (Janvier, 2007).

Amiiformes (Grande & Bemis, 1998, 1999) are represented by one living species, *Amia calva* L., distributed in freshwater habitats in North America. It is the single, living representative of the Halecomorphi, known from marine and fossil taxa dating to the Jurassic.

The ancestral habitat for bony fishes is inferred from the phylogeny and distribution of living and fossil basal actinopterygian taxa to be shallow epicontinental seas and their freshwater margins, including river deltas (Fig. 1). A reconstructed widespread ancestral range was illustrated for a subgroup of halecomorph fishes by Grande and Bemis (1999: fig. 6), who outlined the distribution on a map of present-day geography contrasted with that of the

Table 1. Classification of living aquatic vertebrates (non-tetrapods) or fishes, estimated number of species in each taxon, number of those that live almost exclusively in freshwater, and total number of those that enter freshwater at some time during their life (from Nelson, 2006: 4–5; Stiassny et al., 2004). The last column includes all of the species in the second column, plus those that are migratory (diadromous or euryhaline).

Taxon	No. of species	No. of freshwater species	Total no. of species that enter freshwater
Myxiniformes	70	0	0
Petromyzontiformes	38	29	38
Elasmobranchii	937	24	40
Chimaeriformes	33	0	0
Osteichthyes			
Sarcopterygii			
Coelacanthiformes	2	0	0
Ceratodontiformes	6	6	6
Actinopterygii			
Polypteriformes	16	16	16
Acipenseriformes	27	14	27
Lepisosteiformes	7	6	7
Amiiformes	1	1	1
Teleostei			
Osteoglossomorpha	220	220	220
Elopomorpha	857	6	33
Clupeomorpha	364	79	85
Ostariophysi	7980	7847	7858
Protacanthopterygii	356	127	152
Esociformes	10	10	10
Stomiiformes	391	0	0
Ateleopodiformes	12	0	0
Aulopiformes	236	0	0
Myctophiformes	246	0	0
Lampridiformes	21	0	0
Polymixiiformes	10	0	0
Paracanthopterygii	1340	21	24
Stephanoberyciformes	75	0	0
Zeiformes	32	0	0
Beryciformes	144	0	0
Gasterosteiformes	278	21	43
Synbranchiformes	99	96	99
Perciformes	10,033	2040	2335
Mugiliformes	72	1	7
Atherinomorpha	1552	1304	1352
Scorpaeniformes	1477	60	62
Pleuronectiformes	678	10	20
Tetraodontiformes	357	14	22
TOTALS	27,977	11,952	12,457

Early Cretaceous, 118 Ma. The present-day, disjunct transatlantic distributions are continuous when drawn across Cretaceous epicontinental seaways.

An area cladogram, or areagram, of halecomorphs indicating habitat, marine or freshwater (Fig. 2A), was used by Grande and Bemis (1999: fig. 7) to infer a marine ancestry for the group. This hypothesis results from optimizing habitat or, at the least, inferring that the habitat of the basal taxon is the ancestral habitat. It rests on the assumptions that the habitat has changed (e.g., marine to freshwater), even when there may be no evidence for such a transformation, and that one or the other taxon of a sister group pair may be identified as basal (see Parenti, 2006; Santos, 2007; also see below). Without these assumptions, the ancestral habitat may be reconstructed as epicontinental seas, spanning marine and freshwater habitats (Fig. 2B). Likewise, bichirs in South America and Africa also represent a transatlantic distribution, that is, a remnant of the broader distribution pattern.

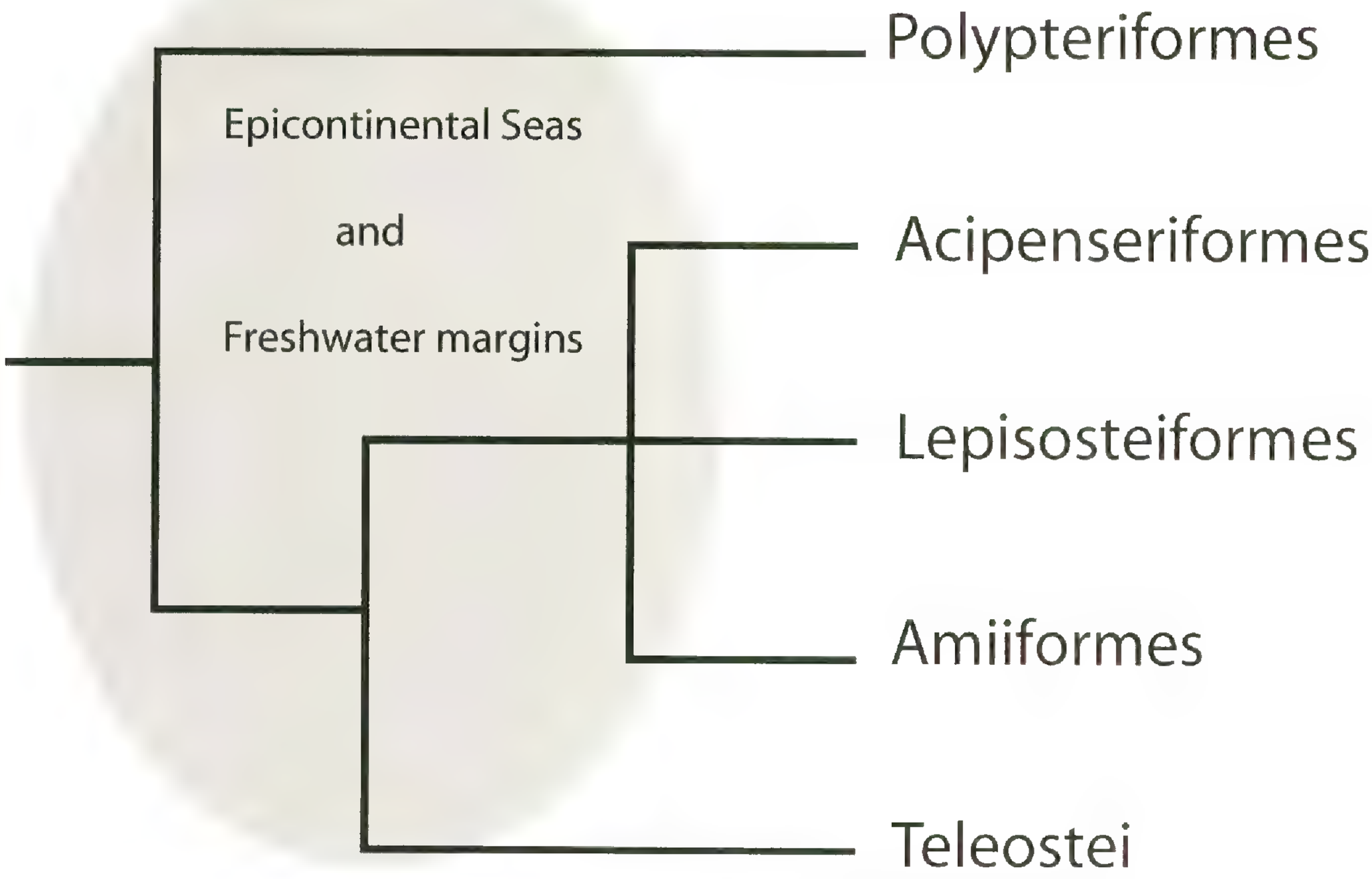

Figure 1. Hypothesized relationships among living actinopterygian clades, following Inoue et al. (2003) and Grande and Bemis (1999). The ancestral distribution is inferred to be throughout epicontinental seas and their freshwater margins.

Paddlefishes reflect transpacific relationships, as do other North American taxa (Grande, 1994a, b).

A lineage with marine and freshwater representatives (Fig. 3A) may be interpreted to have a marine and freshwater ancestral distribution (Fig. 3B). In a likelihood approach to reconstructing ancestral ranges of lineages, Ree et al. (2005: fig. 6c) also reconstruct a widespread ancestral range equivalent to that of Figure 3B when dispersal is considered unlikely, that is, when no a priori process is specified to explain the pattern (see also Ebach, 1999; Santos, 2007). "Ecologically variable ancestors" are analogous to "geographically widespread" ancestors (see Hardy, 2006: 13). (The method of Ree et al. [2005] does not correspond to other cladistic biogeographic methods, in part because it defines an area as a discrete geographic unit, not the organic areas of endemism that are defined by the ranges of the organisms that inhabit them [see Harold & Mooi, 1994].)

Our notion of freshwater and marine fishes comes, in large part, from the existence of speciose taxa that are today nearly all freshwater, such as the Ostariophysi (catfish, characins, minnows, and relatives) or Osteoglossomorpha (bony tongues), or from deep-sea marine orders, such as the Myxiniformes (hagfishes), Chimaeriformes (chimaeras), Stomiiformes (dragonfishes), Aulopiformes (lizardfishes), Myctophiformes (lanternfishes), and so on (Table 1). Osteoglossomorpha is the sister group to all other teleosts, all extant species of which live exclusively in freshwater (Nelson, 1969b, 2006; Table 1). It is not historically a freshwater lineage: marine Paleocene-Eocene fossils, such as the genus †*Brychaetus* Agassiz, are hypothesized to be nested in the osteoglossomorph cladogram in the family Osteoglossidae (Hilton, 2003). The oldest osteoglossomorph fossils are Middle Mesozoic, and Bănărescu (1995: 1162) acknowledged their long history and widespread ancestral distribution by proposing that Osteoglossomorpha is Pangean. Osteoglossomorpha, therefore, has both marine and freshwater representatives and is a freshwater group today because of widespread extinction of marine taxa. If the majority of species in a taxon are marine and just a handful are freshwater, it is often assumed that the group originated in marine habitats and several taxa dispersed to freshwater (e.g., Myers, 1938; Berra, 2001; Tsukamoto et al., 2002). The reverse is also assumed. Such an assumption is not an analysis of biogeographic data, but an implicit and untestable center of origin hypothesis (viz. Croizat et al., 1974).

Both Nelson (1973) and Patterson (1975) optimized habitat on areagrams to infer habitat of origin of osteoglossomorphs and came to different conclusions. For example, Patterson (1975: 162) asserted that "...if the conventional view of the history of

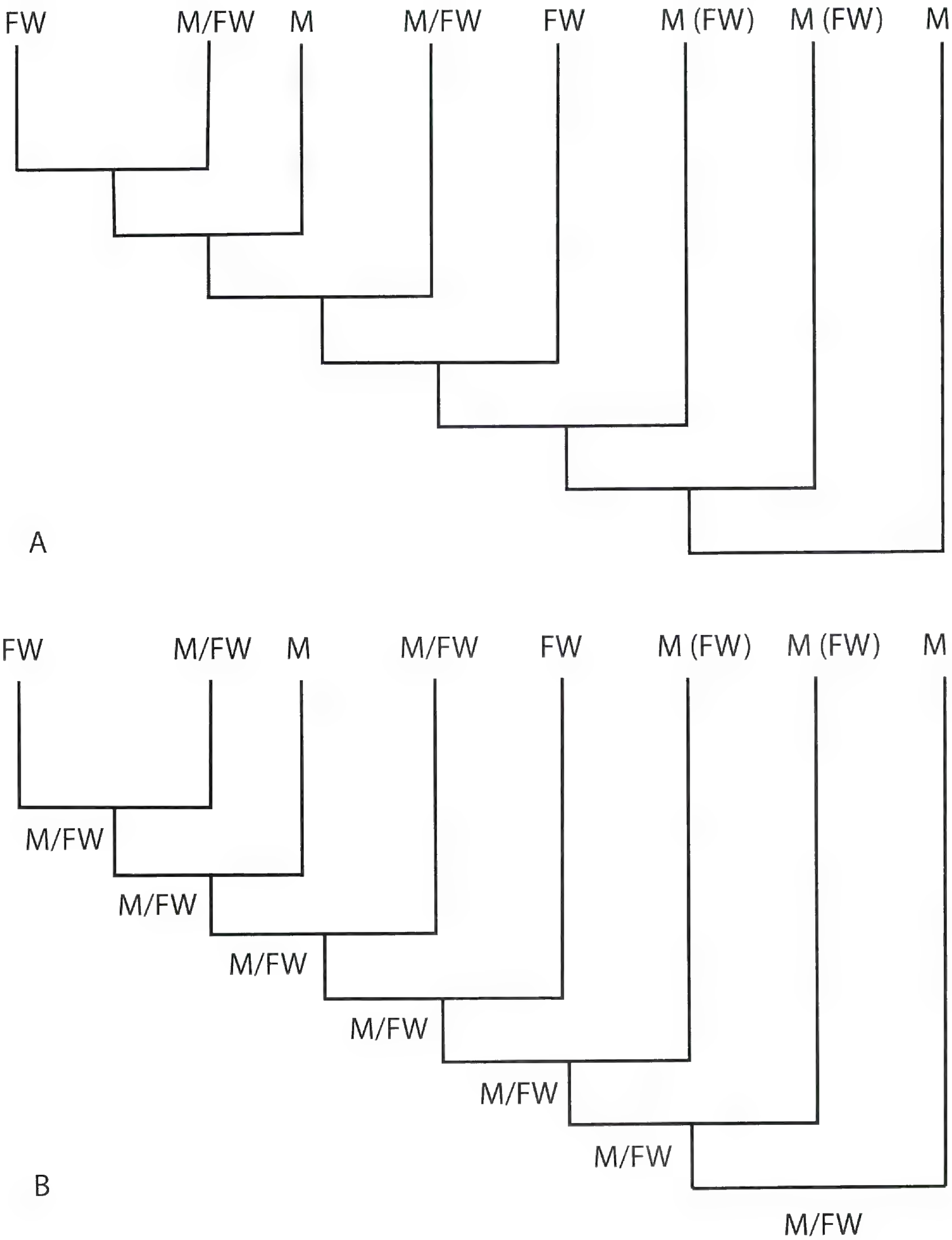

Figure 2. —A. Areagram of halecomorph habitats from Grande and Bemis (1999), who inferred a marine ancestry for the group. M/FW means distribution in marine and freshwater habitats; M (FW) means distribution mostly in marine habitats with some representatives in freshwater. —B. Reconstruction of nodes to infer a widespread marine/freshwater ancestral habitat, interpreted here as epicontinental seas and their freshwater margins.

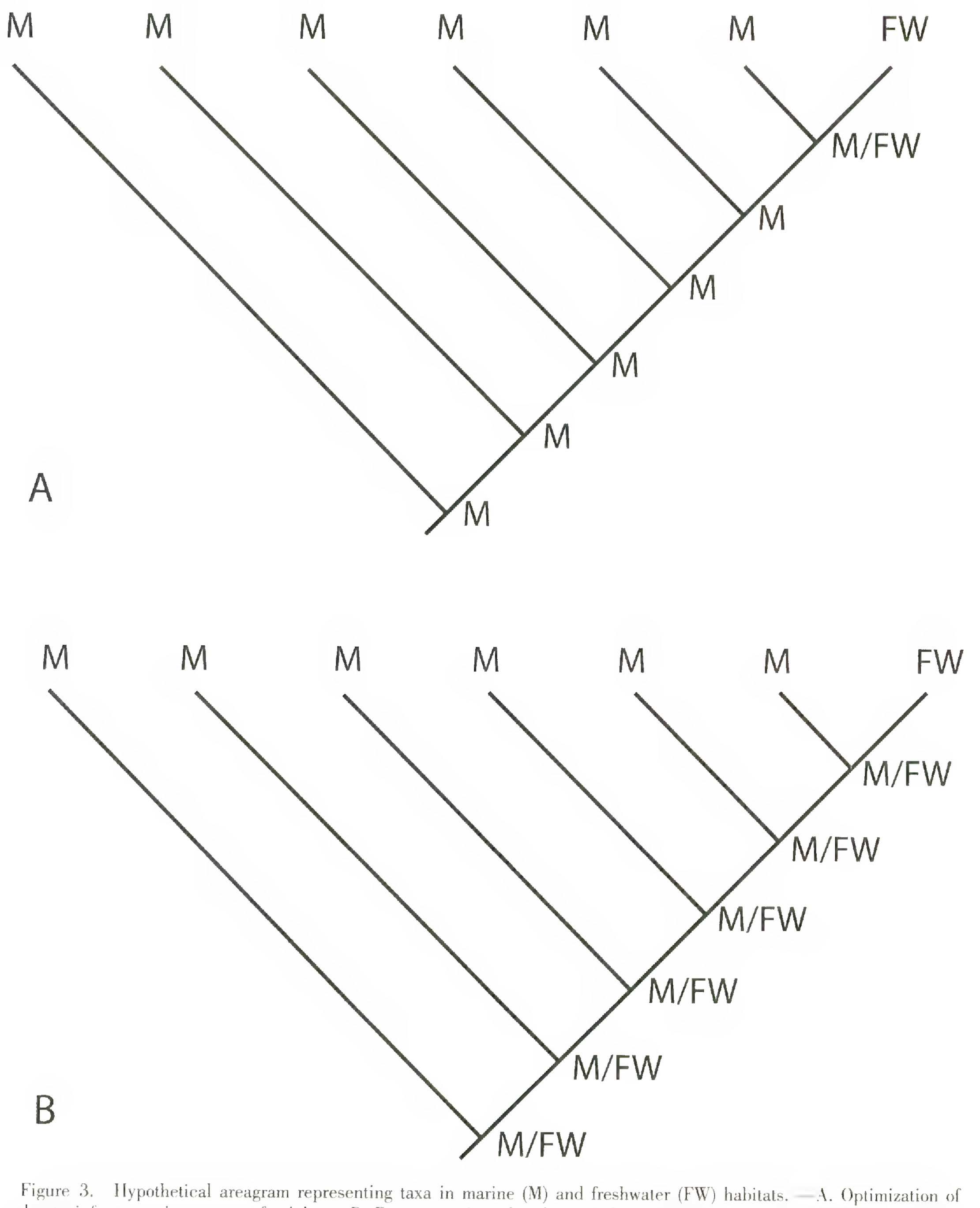

Figure 3. Hypothetical areagram representing taxa in marine (M) and freshwater (FW) habitats. —A. Optimization of nodes to infer a marine center of origin. —B. Reconstruction of nodes to infer a widespread marine/freshwater ancestral habitat.

Gondwana is accepted, marine origin of the Osteoglossomorpha is indicated." Nelson (1973: 9), who read Patterson in manuscript, countered that "... the totality of evidence concerning the relationships and distribution of osteoglossomorphs, both fossil and Recent, indicates that †*Brychaetus* is secondarily marine," that is, that osteoglossomorphs originated in freshwaters.

Even though Nelson later dismissed such arguments (e.g., Nelson, 1974; Nelson & Ladiges, 2001), they persist (see Parenti, 2006). Optimization of habitat on an areagram has been applied broadly to interpret the ecological and distributional history of lineages (see, e.g., Brooks & McLennan, 1991; McLennan, 1994). Optimization of habitat is often justified by the principle of parsimony, yet, in practice, can require

multiple hypotheses of switching between habitats (e.g., Baker, 1978). The distributional history of gobioid fishes, for example, was hypothesized by Thacker and Hardman (2005: 869): "... gobioids arose in freshwater, from a marine ancestor, then returned to marine habitats once or many times."

Whether diadromy or euryhalinity is a primitive or derived life history pattern for fishes has been debated seemingly endlessly (Tchernavin, 1939; Denison, 1956; Patterson, 1975; Griffith, 1987; McDowall, 1988, 1993, 1997a; Johnson & Patterson, 1996; Bemis & Kynard, 1997; Waters et al., 2000; see especially review in Janvier, 2007). The distribution of fishes throughout epicontinental seas, including along their margins, as proposed for basal actinopterygians, is in accord with numerous explanations for the evolution of solely freshwater or marine taxa from widespread diadromous or euryhaline ancestors. Suppression of the marine or of the freshwater life history phase of a migratory lineage could lead to evolution of solely freshwater or marine fishes, respectively, as speculated by Patterson (1975: 168–169). In addition to evolution of development, the roles of extinction and earth history have been recognized: isolation or stranding through changes in sea level or other vicariant events can result in a former euryhaline species being restricted to freshwater, for example (e.g., Choudhury & Dick, 1998; Waters et al., 2000; Heads, 2005a), a phenomenon termed "ecological stranding" (Craw et al., 1999). Widespread extinction of the earth's biota since the Upper Mesozoic has left just remnants of these formerly widespread distributions, "the ring on the bathtub" to paraphrase Heads (1990: 225). Isolation in one habitat or the other by stranding does not require or imply invasion of that habitat (see also Heads, 2005a). Some taxa persist in freshwater, others in marine habitats, and some in both.

Earth History Models

Plate tectonics is the model used most often to interpret global distribution patterns with respect to earth history (e.g., Rosen, 1974; Patterson, 1975; Springer, 1982; Choudhury & Dick, 1998; Grande & Bemis, 1999). The model specifies an Atlantic Ocean expanding since the Mesozoic and supports vicariant explanations for transatlantic taxa, yet leaves biogeographers scrambling for similar explanations, such as migration of allochthonous terranes, for the distribution of transpacific taxa (McCarthy, 2003: 1556). A vicariance explanation of transpacific taxa finds geological support in the still-maligned theory of Expanding Earth, which specifies that the age of the Pacific Ocean is roughly the same as that of the Atlantic and Indian oceans (Shields, 1979, 1983, 1991, 1996; McCarthy, 2003, 2005). Biogeographic sister areas across the Pacific have complementary, matching geological outlines (McCarthy, 2003: fig. 3). That there are competing models of earth history endorses the view that biogeographic patterns should be discovered, then interpreted with respect to these models, rather than the models a priori specifying biogeographic patterns (Ebach & Humphries, 2002).

Minimal divergence times between lineages have been estimated using ages of fossils or a molecular clock and used to test hypotheses of dispersal versus vicariance (e.g., Rosenblatt & Waples, 1986; Sanmartín et al., 2001; Burridge, 2002, to cite just three examples). Minimal estimates from sequence data have been interpreted as absolute, thus calling into question earth history—Plate Tectonics or Expanding Earth—interpretations of distribution patterns (e.g., de Queiroz, 2005). This interpretation has been countered by Heads (2005b), Parenti (2006), and others, continuing a long debate within biogeography that was revived with the advent of molecular techniques (see especially Nelson, 2004). Teleost as well as basal actinopterygian lineages are old enough to be interpreted within earth history models. Fossils of living teleost lineages date from about 150 Ma in the Upper Jurassic, and teleosts as a clade are estimated to be at least of Triassic age (Janvier, 2007; see also Benton & Donoghue, 2007). Differentiation is also ancient: tetraodontiforms, nested within the apomorphic percomorph clade, are represented by fossils from the Upper Cretaceous of Slovenia, Italy, and Lebanon, the oldest of which dates from 95 Ma (Tyler & Sorbini, 1996).

Distribution Patterns of Diadromous Fishes

Historical biogeographic patterns may be complex, and are, by definition, hierarchical. They are characterized by repeated elements that have become the basis of broadly recognized global distribution patterns (Croizat, 1958, 1964) that may be expressed in a classification (Fig. 4): Atlantic, Indian Ocean, (Pacific, Antitropical), or by fragments or composites of these areas. One stated aim of historical biogeography is to develop a standard language or nomenclature for global distribution patterns (e.g., Morrone, 2002) including their fragments. Fragmentation may be caused by extinction, which has left remnants of formerly complete distribution patterns, such as a lone freshwater representative of a taxon once living broadly in marine and freshwater habitats. These remnants have been used to interpret, and misinterpret, the distributional history of taxa (see Heads, 2005a, b; Nelson & Platnick, 1981; Nelson & Ladiges, 1991, 1996; also see below).

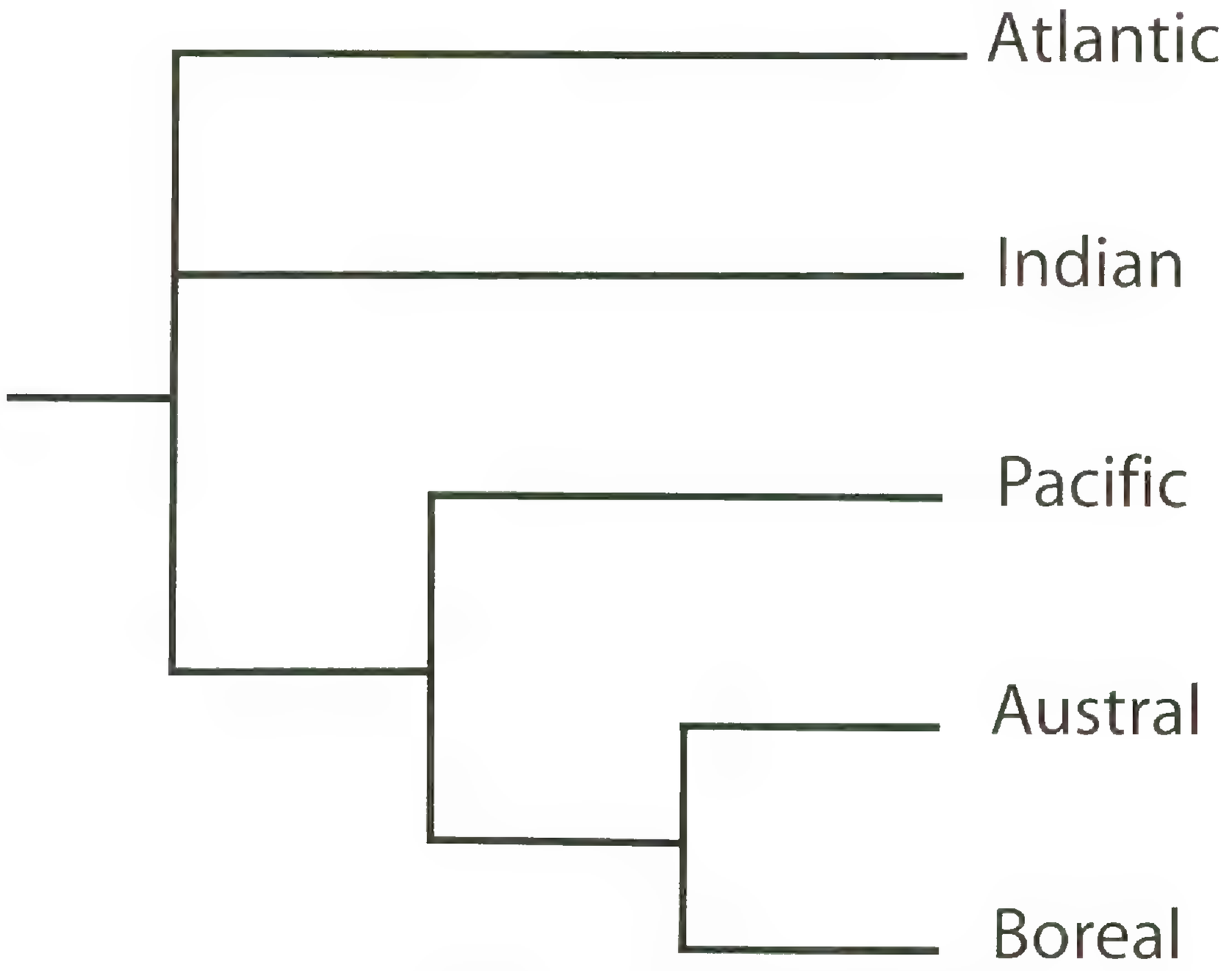

Figure 4. Areagram or classification of Croizat's (1958) and Craw et al.'s (1999) global biogeographic patterns, following Nelson and Ladiges (2001: fig. 8b).

The distribution of antitropical or bipolar taxa has been interpreted with regard to evolution of the Pacific Basin (e.g., Humphries & Parenti, 1986, 1999; Nelson, 1986). In a recent discussion of the evolution of diadromy among fishes of the southern oceans, McDowall (2002: 208) acknowledged but did not discuss bipolarity. He divided fishes of New Zealand into two kinds: those with close relatives throughout the southern oceans, such as lampreys and galaxioids, both groups bipolar, and those that he inferred, a priori, to be part of another global pattern: "Anguillid eels and gobiid [fishes] have *nothing in common* with the southern cool temperate fish fauna regarding historical origins, distributions and relationships. They are probably not Gondwanan and yet, anguillids and gobiids express a series of relationships and distributions between the faunas of eastern Australia and New Zealand that are *essentially the same* as those seen in southern families" (italics added) (McDowall, 2002: 212–213). Sharing a pattern implies sharing a history: I examine the distribution of eels and gobies to ask if they have anything in common with the southern cool fish fauna —in other words, whether they conform to the same global as well as local patterns.

PHYLOGENY AND DISTRIBUTION: EELS

Eels of the genus *Anguilla* are catadromous (Myers, 1949a): adults live in freshwater streams and migrate to marine spawning grounds (Tesch, 1977; Smith, 1989a, b). Phylogenetic relationships among the 19 extant taxa (Fig. 5) were hypothesized using molecular data by two research groups (Lin et al., 2001a, b; Aoyama et al., 2001), and these two sets of analyses were contrasted and compared with morphology (Lin et al., 2005). An areagram of *Anguilla* taxa, with taxon names of Figure 5 replaced by a brief description of distributional limits, is given in Figure 6. Another molecular phylogeny based on whole mitochondrial genome sequences was published by Minegishi et al. (2005).

Anguilla species are associated with all continents, except Antarctica, and do not live in the eastern Pacific or South Atlantic (Berra, 2001). Distribution of anguillid eels has largely been interpreted within a dispersalist paradigm (e.g., Harden Jones, 1968) and, recently, to conflicting ends (Heads, 2005a: 700): Lin et al. (2001a, b) put the center of origin of eels in the southwest Pacific and proposed dispersal eastward across the Pacific and further across the Central American Isthmus to enter the Atlantic Ocean, whereas Aoyama et al. (2001), Aoyama and Tsukamoto (1997), and Tsukamoto et al. (2002) hypothesized dispersal from a center of origin near Borneo, westward through an ancient Tethys Sea to the Atlantic Ocean. Both of these explanations were rejected by Minegishi et al. (2005: 141), who concluded that "...the present geographic distribution [of eels of the genus *Anguilla*] could be attributed to, for example, multiple dispersal events, multidirectional dispersion, or past extinctions, etc."

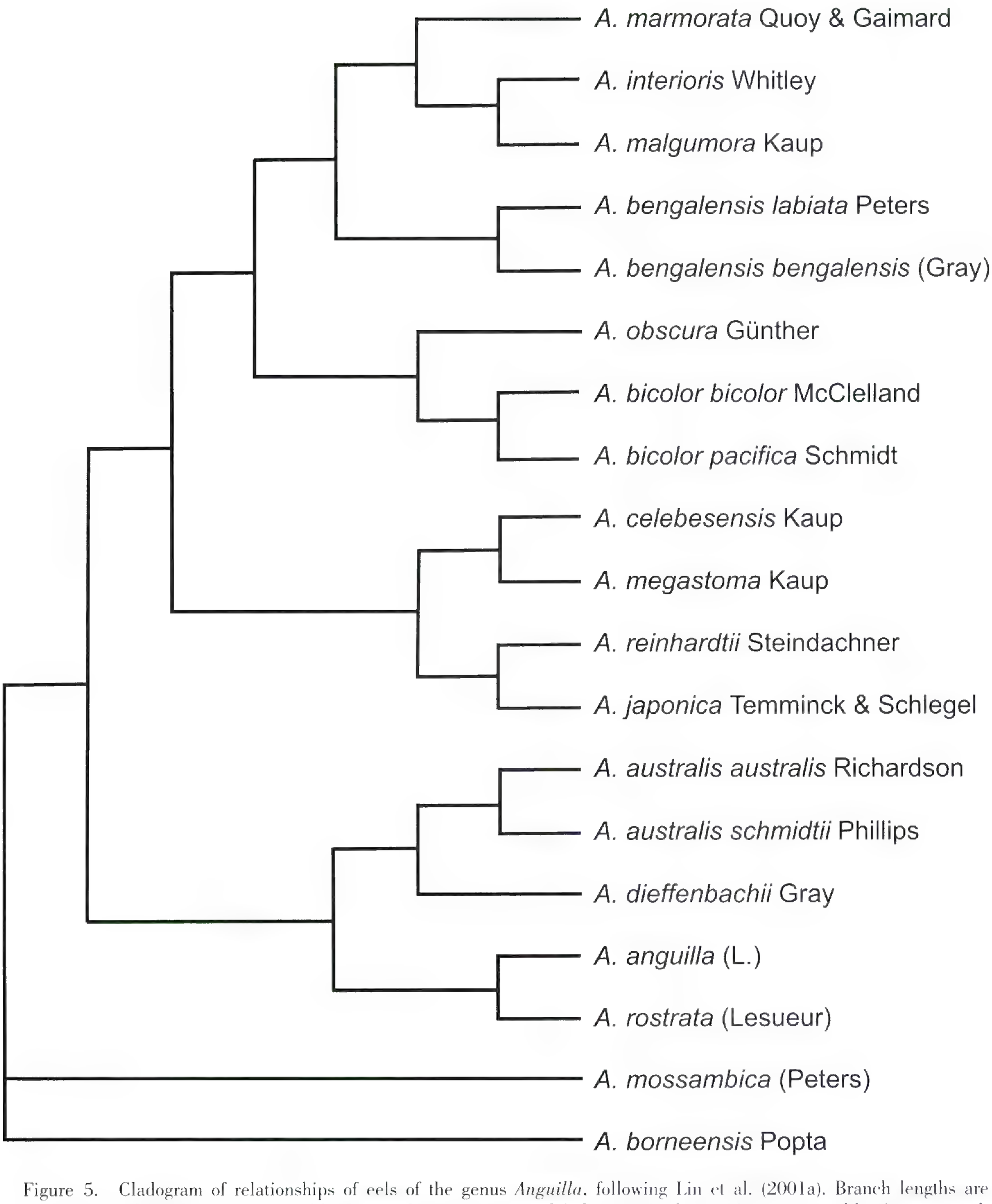

Figure 5. Cladogram of relationships of eels of the genus *Anguilla*, following Lin et al. (2001a). Branch lengths are arbitrary. *Anguilla malgumora*, as used by Lin et al., is a synonym of *A. borneensis*, the species name used by Aoyama et al. (2001) and Minegishi et al. (2005). Spelling of species names and authors follows Eschmeyer's online catalog (http://www.calacademy.org/research/ichthyology/catalog/), accessed on 19 October 2007.

Panbiogeographers have long endorsed the view that the distribution and evolution of eels of the genus *Anguilla* may be interpreted with respect to earth history, specifically evolution of a Tethys Sea biota (Croizat, 1958), and dismiss ad hoc dispersal hypotheses (see Heads, 2005a). Cladistic biogeographers endorse the panbiogeographic view and add relationships among areas, as interpreted from phylogenetic hypotheses, as a framework for further refinement of distributional history patterns. Rather than optimizing areas, the information in the areagram of *Anguilla* taxa (Fig. 6) may be summarized by a variety of methods, such as reduced area–cladograms or paralogy-free subtrees (Nelson & Ladiges, 1996), or by comparing it to the cladistic summary of Croizat's (1958) global biogeographic patterns (Fig. 4). Notable

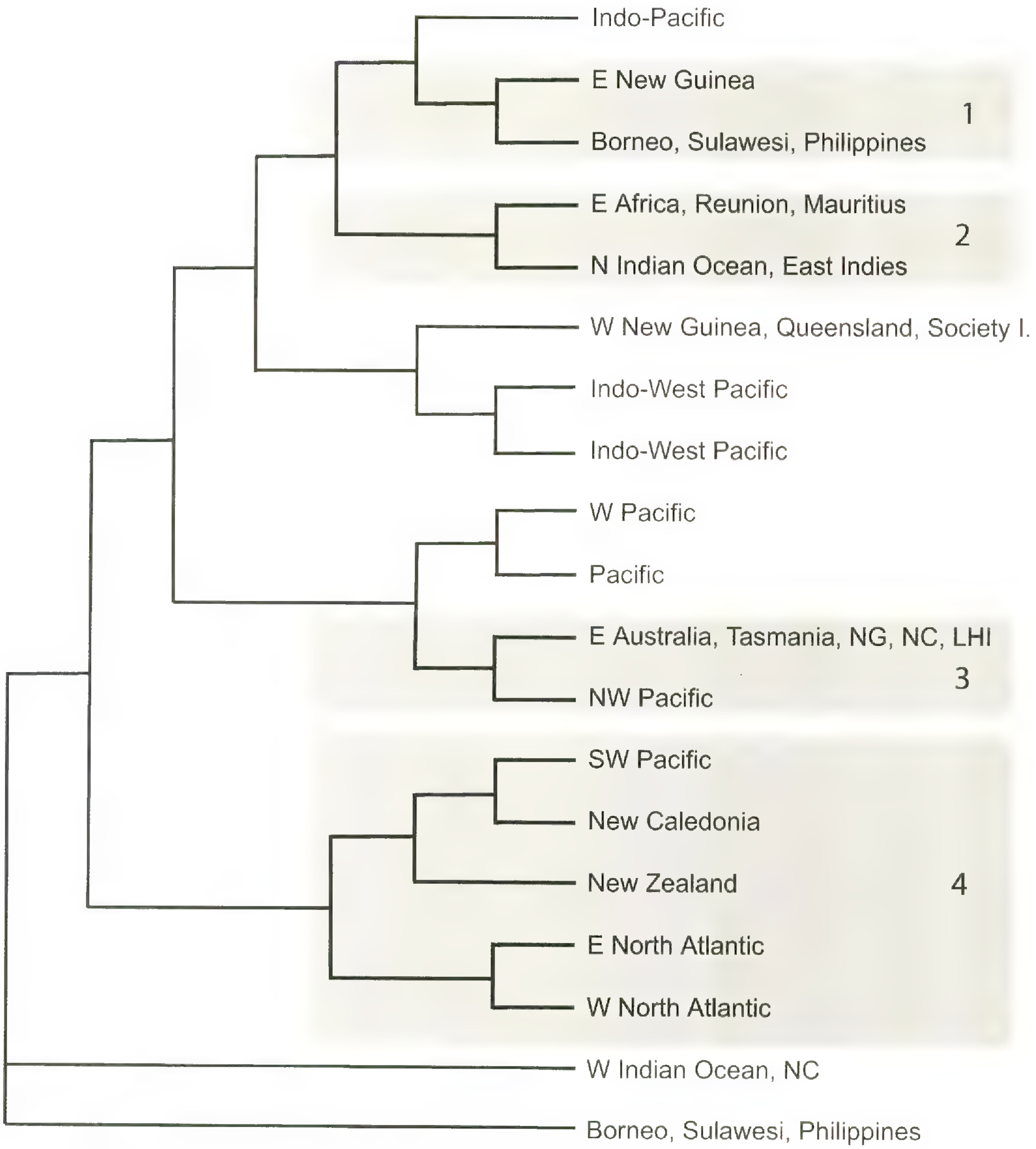

Figure 6. Areagram of *Anguilla* taxa, with taxon names of Figure 5 replaced by brief description of distributional limits. NG = New Guinea, NC = New Caledonia, LHI = Lord Howe Island. Shaded area 1: western margin of Pacific Basin; shaded area 2: Indian Ocean; shaded area 3: antitropical; shaded area 4: antitropical.

in the areagram of *Anguilla* (Fig. 6) are taxa distributed along the western margin of the Pacific Basin (shaded group 1), the Indian Ocean (shaded group 2), antitropical distributions, and antitropical taxa sister to Pacific taxa. Eels demonstrate one of the three-area relationships in the global biogeographic pattern (Fig. 4): Pacific (boreal, austral). Northwest Pacific taxa (boreal) are closely related to those from eastern Australia, Tasmania, New Guinea, and New Caledonia (austral, with additional elements from the western margin of the Pacific Basin; shaded group 3), and these are, in turn, sister to a group of Pacific taxa. Another clade is also antitropical: the North Atlantic sister species (boreal) are closely related to a group of southwest Pacific, New Zealand, and New Caledonian taxa (austral; shaded group 4). Freshwater eels of the genus *Anguilla* have not been characterized as antitropical or bipolar, as antitropicality of fishes is seen largely as a phenomenon of strictly marine taxa (e.g., Nelson, 2006: 12). These remnants of antitropicality would likely not be identified without a hypothesis of phylogenetic relationships or without

the aim of identifying general patterns, rather than hypothesizing individual distributional histories.

Are anguillid eels ancient, like other antitropical taxa? Perhaps. A molecular clock estimate of 20 Ma for the beginning of *Anguilla* speciation was rejected by Minegishi et al. (2005: 141) as an underestimate because of fossil evidence: anguilliforms date from the Upper Cretaceous (113–119 Ma) and the genus *Anguilla* from the Eocene (50–55 Ma) (Patterson, 1993), both minimum estimates of the age of these lineages.

PHYLOGENY AND DISTRIBUTION: GOBIES

Gobioid fishes comprise over 2000 species distributed broadly in pantropical and temperate freshwater, estuarine, and marine aquatic habitats (Nelson, 2006). Because the vast majority of gobioids live in marine waters, they have been characterized as a marine group (e.g., Myers, 1938; Berra, 2001). Several subgroups, such as *Gobiomorphus* Gill, and the gobiid subfamily Sicydiinae are diadromous (e.g., Parenti & Maciolek, 1993; Keith, 2003). Phylogenetic analysis of sicydiine gobies and their relatives at the generic level (e.g., Parenti, 1991; Parenti & Thomas, 1998) has been offered as evidence that the repeated, recognizable biogeographic areas for gobioids are ocean basins, not continents (see also Springer, 1982; Gill in Lundberg et al., 2000).

Hypotheses of relationships among gobioids (e.g., Thacker & Hardman, 2005: fig. 1) are preliminary, yet, when combined with distribution, display remnants of global patterns (Fig. 4): Rhyacichthyidae, the sister group to all other gobioids, lives in the tropical western Pacific (Berra, 2001: 458), whereas Odontobutidae, sister to the remaining gobioids, exhibits a remnant boreal distribution, living in freshwaters of the northwest Pacific (Berra, 2001: 460). The southeast Australian and New Zealand *Gobiomorphus*, a goby genus that McDowall (2002: 213) characterized as "probably not Gondwanan," is interpreted as having a remnant of an austral distribution; it is sister to a large gobioid lineage distributed throughout the pantropics. The fossil record of gobiids is scant; they date from at least the Middle Tertiary (Patterson, 1993).

Endemism of gobioids on islands or island groups is high, notably throughout the Indo-Pacific (Maugé et al., 1992; Parenti & Maciolek, 1993, 1996; Larson, 2001; Donaldson & Myers, 2002). Endemism throughout the marine realm is underappreciated in large part because marine species are defined traditionally as widespread (e.g., Gill & Kemp, 2002, fishes; Meyer et al., 2005, gastropods). Endemism may be underrecognized at both lower (species) and higher (genera) taxonomic levels. The lack of genetic structure among populations of the five freshwater or amphidromous gobiid fish species living throughout Hawaiian streams (e.g., Chubb et al., 1998) prompted McDowall (2003) to conclude that they must have colonized Hawaii by long-distance, chance dispersal. Alternative explanations are plausible. Four of the five species are endemic to the Hawaiian Islands, as McDowall (2003: 705) acknowledged. Traditional island biogeography tells us that what inhabits an island or island group is what is able to physiologically and physically stand a long ocean voyage from the mainland (MacArthur & Wilson, 1967), although islands, as well as continents, are inhabited today by the remnants of once more diverse faunas. Islands and coastal zones support life that is able to withstand the wide swings in salinity, temperature, and so on (see also Heads, 2005a), and chief among that life is the diadromous or euryhaline taxon.

CONCLUSIONS

Biogeography of diadromous fishes is not about whether they are capable of surviving in the seas. They are, at least during some phase of their life history. It is not about whether diadromous fishes have marine or freshwater ancestors because all fish lineages have both. Biogeography of diadromous fishes is like that of all other taxa: a search for a pattern of relationships among endemic areas that conforms to or differs from general distributions of taxa worldwide. Eels and gobies, phylogenetically disparate and distinct diadromous taxa, reflect global biogeographic patterns. One can acknowledge this congruence, this conformity to a pattern, or one can ignore it. Predictions that may be made about distribution of diadromous taxa based on their life history are not necessarily corroborated by their phylogeny or their distribution patterns. The answer to "Why migrate?" is straightforward when earth history is considered: migration evolves as the earth evolves (see also Wolfson, 1948; Croizat, 1958; Heads, 2005a). Migration of diadromous species is "the result...not the cause" of their evolution (Wolfson, 1948: 30; see also Grehan, 2006). When interpreting the evolution of diadromy in fishes, or of any migration pattern, the relationship between phylogeny and distribution must be considered along with ecological patterns. Ecology, phylogeny, and distribution are inseparable (see also Heads, 1985).

The ability to live in both freshwater and marine habitats is interpreted as an ancestral life history pattern for fishes. The evolution of diadromy is tied to the evolution of ocean basins. Optimizing habitat on the nodes of an areagram to invoke an ancestral habitat, here marine or freshwater, is equivalent to

invoking a center of origin for areas. A repeated, general pattern among taxa that are not closely related and/or that differ in ecological requirements is most parsimoniously explained by the same events. There should be no separate global historical biogeography for marine and freshwater fishes. Proposing separate explanations for each prevents discovery of general patterns.

Today, we see but a remnant of the past distribution of life on earth. Global biogeographic patterns are the organizing framework for interpreting distributional and ecological history of both marine and freshwater taxa. Recognizing that anguillid eels have remnants of antitropical patterns, for example, is a first step toward accepting that their distribution may be classified along with that of other taxa, rather than a priori requiring a unique explanation.

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CONGRUENCE AND CONFLICT BETWEEN MOLECULAR AND REPRODUCTIVE CHARACTERS WHEN ASSESSING BIOLOGICAL DIVERSITY IN THE WESTERN FANSHELL *CYPROGENIA ABERTI* (BIVALVIA, UNIONIDAE)[1]

Jeanne M. Serb[2] and M. Christopher Barnhart[3]

ABSTRACT

Organisms with complex life histories and unusual modes of genome inheritance can present challenges for phylogenetic reconstruction and accurate assessment of biological diversity. This is particularly true for freshwater bivalves in the family Unionidae because: (1) they have complex life cycles that include a parasitic larva and obligate fish host; (2) they possess both a male and female mitochondrial genome that is transmitted through doubly uniparental inheritance (DUI); and (3) they are found in riverine habitats with complex hydrogeological histories. Examination of mitochondrial DNA (mtDNA) sequences, conglutinate morphology, and host fish compatibility of the western fanshell *Cyprogenia aberti* (Conrad, 1850) revealed significant character variation across its range. Although variation was correlated among the different data sets and supports discrete groups, these groups did not always correspond to geographically isolated populations. Two discrete mtDNA clades exist sympatrically within most *C. aberti* populations, and these same clades are also diagnosed by at least one morphological character, egg color. The surprisingly high genetic distance (14.61%–20.19%) between the members of these sympatric clades suggests heritance infidelity of the two different mitochondrial genomes. This hypothesis was tested and falsified. More general patterns in geography were observed in host fish compatibility. Populations of *C. aberti* from the major river systems differed in their ability to utilize fish species as hosts. These differences in reproductive traits, which are presumably genetically based, suggest that these populations are not ecologically exchangeable with one another and represent biological diversity not previously recognized within *Cyprogenia* Agassiz, 1852.

Key words: Central Highlands, coevolution, *Cyprogenia aberti*, double uniparental inheritance, mitotype, Unionidae.

Comparison among associated historical patterns is a powerful tool in evolutionary biology. Such patterns include organisms and geographic areas in vicariance biogeography (Rosen, 1978), the co-speciation of hosts and parasites (Hafner & Nadler, 1988), and congruence between gene trees and species trees (Goodman et al., 1979). Areas, organisms, and genes are analogous because each has a history that can be reconstructed via phylogenetic methods. Likewise, the affiliations between these patterns are analogous, for each pair has one entity that is connected to, and may track, the other because of causal interaction through evolutionary history (Page, 1993; Page & Charleston, 1998). Comparing lineage patterns in an evolutionary association can provide insight into the biology of understudied organisms, direct research by focusing attention on meaningful patterns, reveal hidden biological diversity, and help identify the processes and mechanisms involved in speciation.

Although evolutionary associations may provide mechanisms for speciation, lineages (organism vs. gene vs. area vs. host) may or may not show congruent patterns. Pattern differences may reflect a unique history at one level that did not influence lineage diversification at another. An example of such incongruence would be the failure of a parasite to track its host after a host-switching event (Page, 1994). However, when data from different evolution-

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ary associations converge on a single pattern, it is a strong indication that the pattern represents the true species tree (Page & Charleston, 1998).

North American freshwater mussels in the family Unionidae offer an unusual opportunity to examine multiple associations simultaneously, because the greatest diversity occurs in areas with complex hydrogeological histories, their life cycle includes an obligate parasitic stage, and they possess an unusual mode of mitochondrial inheritance. The first two factors appear to play a major role in unionid speciation (Kat, 1984) and may be useful when assessing biological diversity (Roe & Lydeard, 1998; King et al., 1999; Roe et al., 2001). In contrast, mitochondrial genes are often used in phylogenetic reconstruction because most models of mitochondrial DNA (mtDNA) evolution consider neutral evolution and purifying selection as the major processes that shape sequence divergence. Thus, mtDNA variation should track species history. However, alterations in mitochondrial inheritance could affect phylogenetic inferences from these genes, making them incongruent with other lines of evidence (Hoeh et al., 1996, 1997).

The life cycle of North American unionids includes a larval stage that is obligately parasitic on a vertebrate host (for a general description of unionid life history, see Coker et al., 1921; McMahon, 1991). The parasitic larvae, called glochidia, are brooded inside the female mussel in a modified portion of the gill. Once mature, the glochidia must attach to a host to complete development. Generally, the host is a fish, and the glochidia encyst in the gill or fin epithelium of the appropriate host species. Unionids are typically able to utilize only a limited number of species as hosts. Glochidia that attach to a compatible host are encysted by migrating epithelial cells and subsequently transform into the juvenile stage (Arey, 1932; Rogers-Lowery & Dimock, 2006). Glochidia that attach to incompatible (non-host) species either fail to be encysted or are subsequently sloughed before transformation is complete (Arey, 1932; Meyers et al., 1980; O'Connell & Neves, 1999; Rogers & Dimock, 2003).

Relationships between mussels and host species may influence speciation rates and/or provide information for phylogenetic reconstruction. The degree of host specificity varies among unionid taxa (Hoggarth, 1992). Some host-parasite relationships are highly restrictive, so that a mussel species will only transform on a specific (sympatric) population within a host species range (Rogers et al., 2001; Eckert, 2003). In these instances, lineage associations between mussel parasite and fish host should be highly correlated.

Females of some host-specific mussel species (e.g., *Lampsilis cardium* (Rafinesque, 1820), *Villosa iris* (Lea, 1829)) attract particular hosts by producing lures that mimic prey items of intended host (see Kat, 1984; Barnhart et al., 2008 and references within). Lures can be part of the female's mantle tissue or may take the form of conglutinates, aggregations of larvae (glochidia) and unfertilized eggs that are released from the female. When a fish attacks the conglutinate, it comes in direct contact with the glochidia, which increases the probability of infection. At the same time, the lure limits the diversity of fish that are likely to come into contact with the glochidia of the species and, therefore, affects the range of potential hosts. The evolution of lures may thus affect the evolution of host specificity. For example, change in display timing may alter the level of attractiveness to a host. Kat (1984) and Graf (1997) suggest that this change in fish hosts might result in the formation of new unionid species via sympatric means.

Another evolutionary association in unionids occurs between the organism and its geographic range (area). Similar to freshwater fishes and crayfishes, unionids exhibit distinct faunal assemblages and high endemism among river basins (Neves & Widlak, 1987; Vaughn, 1997). The greatest unionid diversity is found in ancient rivers with dynamic hydrogeological histories (Lydeard & Mayden, 1995; Neves et al., 1997). Many of these rivers have undergone reorganization during the cyclic glacial advances and retreats of the Pliocene and Pleistocene (e.g., Donn et al., 1962; Melhorn & Kempton, 1991) or experienced episodic tilt-block tectonics (e.g., Cox, 1994) during the Pleistocene and Early Holocene. These geologic events have profound evolutionary effects by restricting gene flow, causing allopatric speciation through vicariance, or creating opportunities for rapid radiations and secondary contact zones between once-isolated populations through stream-capture events (Roe et al., 2001; Kozak et al., 2006). Thus, understanding the historical geology of the drainages in which the study organisms occur is important when interpreting phylogenetic reconstruction. Phylogenetic patterns should reflect the hydrogeological history of the drainage basins when the organism is restricted to aquatic environments and has limited dispersal capabilities. There will be some exceptions to this prediction. In a dynamic environment with multiple, rapid cladogenetic events, area relationships and gene lineages may be in conflict with one another due to incomplete lineage sorting of ancestral polymorphisms. Incongruence between the unionid and area history also may occur when mussel species use hosts with high vagility.

Finally, unusual modes of genomic inheritance can complicate phylogenetic analysis and interpretation where the gene tree may not reflect the species tree.

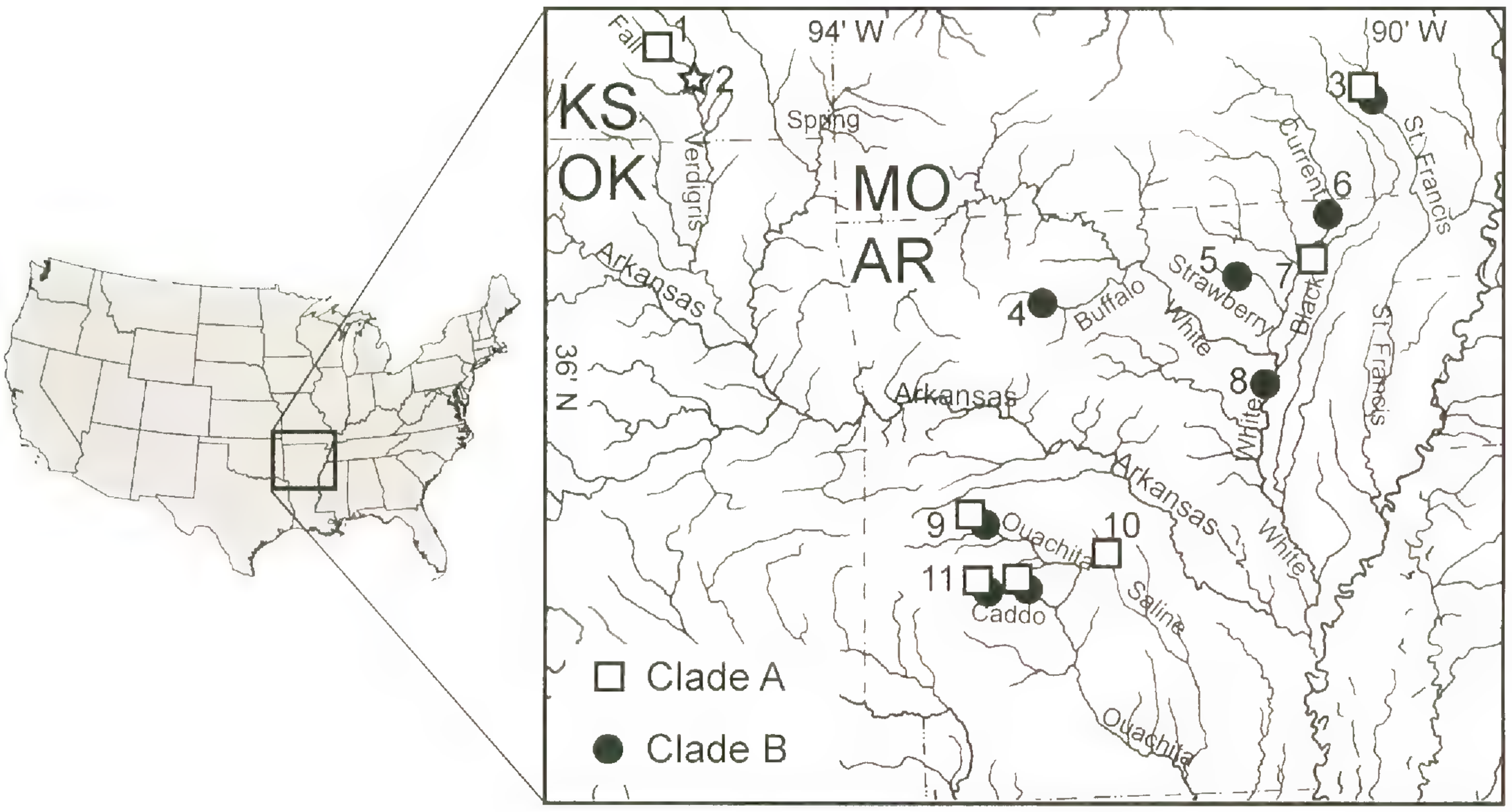

Figure 1. Distribution map of sampled *Cyprogenia aberti* localities. Squares and circles represent the two major genetic clades recovered in the phylogenetic analyses, clade A and clade B, respectively (see Fig. 3). A star represents the collection site on the Verdigris River that was used in a host identification study by Eckert (2003), but was not included in this phylogenetic analysis (but see phylogenetic analysis in Serb, 2006). Specific locality information is provided in Appendix 1 and Serb (2006). Collection sites are labeled by river name and number: Fall (1), Verdigris (2), St. Francis (3), Buffalo (4), Strawberry (5), Current (6), Black (7), White (8), Ouachita (9), Saline (10), and Caddo (11). States are labeled with abbreviations: Arkansas (AR), Kansas (KS), Missouri (MO), and Oklahoma (OK).

The Unionidae and two other bivalve families (Mytilidae and Veneridae) possess two different mitochondrial genomes (mitotypes) that are associated with gender (F for female and M for male) (Zouros et al., 1992, 1994; Hoeh et al., 1996, 1997; Liu et al., 1996; Passamonti & Scali, 2001). These sexual mitotype lineages remain separate via doubly uniparental inheritance (DUI) (Skibinski et al., 1994; Zouros et al., 1994), where the maternal mitochondrial genome (F-mitotype) is inherited by both male and female progeny from the mother, while the paternal mitochondrial genome (M-mitotype) is only transmitted from father to male offspring and housed in the male gonads. The resulting heteroplasmy is not necessarily limited to males. In both laboratory crosses and natural populations, low levels of DUI infidelity have been documented in *Mytilus* L. species (family Mytilidae) (Zouros et al., 1994; Stewart et al., 1995; Garrido-Ramos et al., 1998). This event is a role reversal of sexual mitotypes by either masculinization of the F-mitotype (M^f) or feminization of the M-mitotype (F^m) (as described by Hoeh et al., 1997). The result is that two lineages of the functional mitotype (e.g., F and F^m) co-exist in a population. These events can confound the interpretation of phylogenetic analyses when non-orthologous genes (F vs. F^m) are inadvertently compared (see Hoeh et al., 1996, 1997), so that the resulting gene tree would not represent the species tree.

Cyprogenia aberti, A Case Study

The western fanshell *Cyprogenia aberti* (Conrad, 1850) is the only congener of the federally endangered *C. stegaria* (Rafinesque, 1820) (U.S. Fish & Wildlife Service, 1990). These two species occur in the Central Highlands of North America, with *C. aberti* found in the Interior Highlands west of the Mississippi River and *C. stegaria* in the Eastern Highlands east of the Mississippi River. The Central Highlands are drained by high-gradient, clear streams and are isolated by low-gradient habitats that are typically silt loaded. The two Highland provinces contain a diverse, well-studied aquatic fauna and many endemic taxa, including fishes (Wiley & Mayden, 1985; Mayden, 1987, 1988), amphibians (Routman et al., 1994), and crayfishes (Crandall & Templeton, 1999).

Cyprogenia aberti is endemic to rivers draining the Ozark Plateau and the Ouachita Highlands of the Interior Highlands. These include upland portions of the Arkansas, Ouachita, White, and St. Francis river systems in Arkansas, Kansas, Oklahoma, and Missouri (Fig. 1). Many *C. aberti* populations are isolated by geography between rivers and within river systems. For example, populations in streams of the upper Arkansas, Black, and White rivers are all separated from one another by the lower-elevation, low-gradient portions of these systems.

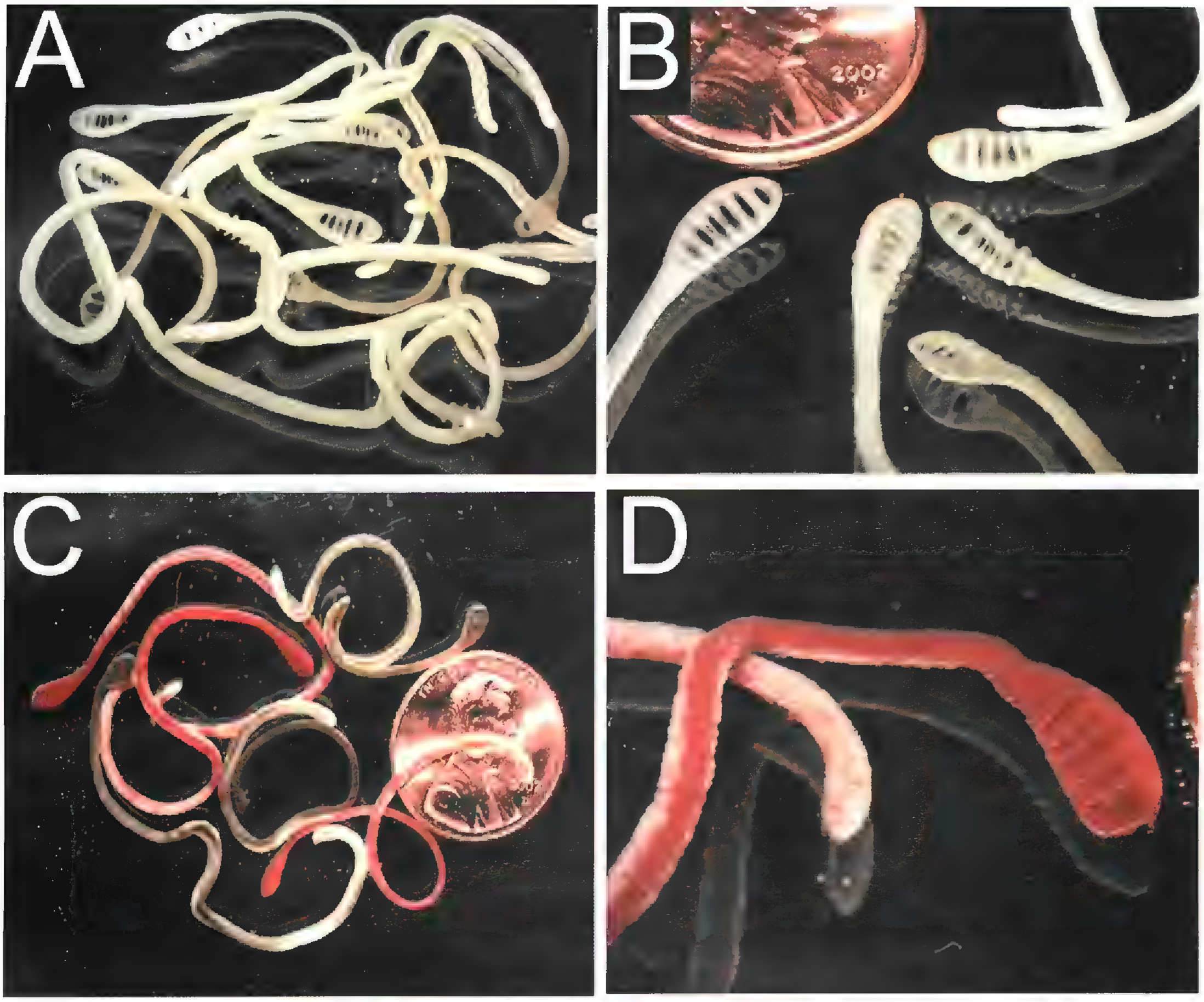

Figure 2. Variation in conglutinate morphology. Color of the unfertilized eggs and the structure of conglutinate lure differs among *Cyprogenia aberti* females. —A–B. White conglutinates were produced by a female collected in the Verdigris River, Kansas. Similar conglutinate morphology is produced by Fall River females. —C–D. Red and brown conglutinates were produced by two sympatric females collected in the St. Francis River, Missouri.

Geography would appear to be the best predictor of how populations of *Cyprogenia aberti* would be related to one another due to their disjunct distribution. However, recent genetic investigations of *C. aberti* using mtDNA sequence data indicate that geography only partly explains the phylogenetic pattern (Serb, 2006). Although the phylogenetic clades correspond to drainage, this pattern was replicated within *C. aberti*, where two genetically distinct sympatric lineages were identified within the Ouachita River system as non-sister clades (Serb, 2006). If the mitochondrial gene tree accurately describes the species tree, these data suggest that there are as many as five distinct evolutionary lineages within *C. aberti* (Serb, 2006). To test the accuracy of this biodiversity assessment, we compared molecular, geographic, and reproductive characters.

Reproductive traits employed in attracting a fish host, such as lure morphology, presumably have a genetic basis and may provide additional data for determining the species tree. In *Cyprogenia*, conglutinate coloration varies among species and populations. For example, *C. stegaria* typically produces red worm-like conglutinates (Jones & Neves, 2002). Among populations of *C. aberti*, red conglutinates have been documented from the St. Francis River (Chamberlain, 1934), while white conglutinates were observed in the upper Arkansas River (Barnhart, 1997) (Fig. 2A, B). Most recently, Eckert (2003) identified populations in the Ouachita and St. Francis rivers that contain females that produce either red or brown conglutinates (Fig. 2C, D). Conglutinate color is the result of pigmentation in the sterile (structural) eggs (Barnhart, 1997; Eckert, 2003). The red and brown forms are pale when immature but darken during development (Barnhart, pers. obs.).

The goal of this study is to better understand the types of data needed to accurately assess biological diversity in organisms with complex evolutionary histories. We explore how three evolutionary associ-

ations of a North American unionid, *Cyprogenia aberti*, relate to one another: reproductive traits associated with host preference, geographic distribution, and mitochondrial gene variation. Specifically, we compare the geographic distribution and reproductive traits with a mitochondrial gene tree derived from previous work (Serb, 2006).

Materials and Methods

Thirty-one individual specimens of *Cyprogenia aberti* representing 11 different localities were collected across its extant range in Arkansas, Kansas, and Missouri. Individuals are identified with a unique museum number and river location. The following river and geographic notations are used. "White" refers to the upper White River above the confluence of the Black River and its tributaries (Strawberry, Current, and Buffalo rivers). The upper St. Francis River is included in the White designation, because the two rivers have similar fish faunas attributed to interchanges between headwaters (Cross et al., 1986). "Arkansas" refers to the upper Arkansas River and its tributaries in Kansas (including the Fall and Verdigris rivers). "Ouachita" refers to the Ouachita, Caddo, and Saline rivers in the Ouachita Highlands. Rivers of the Interior Highlands include all drainages of the Ozark Plateau and Ouachita Highlands. Figure 1 is a map of rivers and localities in this study.

DNA Sequencing

Genomic DNA was extracted from the *Cyprogenia aberti* specimens and six outgroup taxa also within the Unionidae: *C. stegaria* (eastern fanshell), *Dromus dromas* I. Lea (dromedary pearly mussel), *Lampsilis ornata* Conrad (southern pocketbook), *L. siliquoidea* Barnes (fatmucket), *Obliquaria reflexa* Rafinesque (three-horn wartyback), *Ptychobranchus fasciolaris* Rafinesque (kidneyshell) (Appendix 1). Mantle tissue along the shell was used in standard phenol/chloroform extraction with ethanol precipitation (Roe & Lydeard, 1998) to reduce the probability of non-orthologous mitotype contamination from the male gonad.

DNA amplification and sequencing of two mitochondrial gene portions were performed as described in Serb (2006). A 600-bp region of the first subunit of cytochrome oxidase *c* gene (CO1) was amplified and sequenced using primers LCO1490 and HCO2198 (Folmer et al., 1994) or a modified HCO2198 primer (Graf & Ó Foighil, 2000). A 700-bp region of the 5′ end of the first subunit of the NADH dehydrogenase gene (ND1) was amplified and sequenced using primers Leu-uurF and NU-12073 (Serb et al., 2003). Purified polymerase chain reaction (PCR) products were used as a template for cycle sequencing reactions with the ABI Prism Big Dye Terminator kit (Applied Biosystems, Foster City, California). Sequencing reactions were visualized on an ABI 3100 automated sequencer.

A male mitotype from *Cyprogenia aberti* was isolated and sequenced for phylogenetic analysis. Testis tissue was dissected from an ethanol-preserved *C. aberti* male from the Black River, Missouri (see Appendix 1). Total RNA was isolated from gonad tissue using TRIZOL (Invitrogen, Carlsbad, California), followed by phenol/chloroform extraction and ethanol precipitation. RNA was used as a template for complementary DNA synthesis in reverse transcriptase-PCR with a poly-T primer. Complementary DNA was quantified and used in subsequent PCR to amplify the male mitotype copy of CO1. A 550-bp region of the male copy of CO1 was amplified and sequenced using primers UNIOMCOIF (Curole, 2005) and HCO2198, by means of the following thermal cycling conditions for 34 cycles: denaturing at 94°C for 40 seconds, annealing at 45°C for 60 seconds, and extension at 72°C for 90 seconds.

Conglutinate Phenotypes

Six to eight gravid females of *Cyprogenia aberti* were collected from the Fall (Arkansas system), St. Francis, and Ouachita rivers. These females were brought back to the laboratory, where conglutinate release was induced by raising water temperature from 5°C to 20°C over a period of seven hours. Maturity of glochidia and conglutinate color were determined, which allowed for individuals to be designated as known phenotypes. All animals were preserved in 95% ethanol. DNA sequencing was performed as described above for correlation with conglutinate phenotype.

Phylogenetic Analyses

All double-strand DNA sequences were visually aligned unambiguously and were converted into amino acid sequence to check the accuracy of the nucleotide sequence in the data editor BioEdit (Hall, 1999). For each protein-coding gene, an evolutionary model was determined using the log likelihood ratio test (LRT) as implemented in Modeltest version 3.0 (Posada & Crandall, 1998). Pairwise sequence divergences were calculated between all taxa using the appropriate evolutionary model. Mean sequence divergence was calculated within and between phylogenetically defined clades.

Aligned sequences were analyzed phylogenetically under two optimality criteria: maximum parsimony (MP) using PAUP* version 4.0b10 (Swofford, 2002) and Bayesian using the program MrBayes version 3.0 (Huelsenbeck & Ronquist, 2001). All MP analyses employed a heuristic search of 100 random-order addition of taxa and tree bisection-reconnection (TBR) branch swapping. Only minimum-length trees were retained and zero-length branches were collapsed. Support for the individual nodes of the resulting phylogenetic hypotheses was assessed by bootstrap values using the FAST step-wise addition option (2000 replicates) in PAUP*, and decay index values (Bremer, 1994) were calculated with TreeRot version 2c (Sorenson, 1999). Outgroup taxa within the Unionidae were chosen based on a molecular phylogeny of the tribe Lampsilini using CO1 and 16S ribosomal RNA (rRNA) sequence data (Roe, 1999). Trees were rooted using the species *Lampsilis ornata*. MP analyses comparing M- and F-mitotypes were based on the CO1 gene alignment. Trees were rooted using the M-mitotype copy of CO1 from *L. teres* Rafinesque (GenBank accession number AF406794).

A Bayesian analysis of the concatenated mitochondrial genes was conducted using six data partitions: first, second, and third codon positions for two protein-coding genes. Four Markov chain Monte Carlo (MCMC) simulations were run simultaneously using a random starting tree for 3×10^6 generations to estimate the topology and posterior probability for node support. Trees were sampled every 100 generations, and the number of trees discarded (burn-in) was determined by plotting the log likelihood scores of all saved trees versus generation time. Only trees with likelihood scores after stationarity was achieved were retained for inclusion in the consensus tree. Node support was determined by the frequency at which a particular clade occurred within all trees retained after the burn-in trees were discarded.

Results

Conglutinates from Fall River (1, cf. Fig. 1) *Cyprogenia aberti* females were all white in color, similar to previous observations of specimens from the upper Arkansas system (Fig. 2A, B; Barnhart, 1997; Eckert, 2003). Females from the St. Francis (3, Fig. 1) and Ouachita (9, Fig. 1) rivers produced either red or brown conglutinates (Fig. 2C, D).

Sequence alignment of the CO1 and ND1 gene portions yielded 1126 bp, containing 402 polymorphic sites, 261 of which were phylogenetically informative under MP. The MP analyses conducted on these concatenated sequences resulted in 7476 equally most parsimonious trees (MPT) of 734 steps (Fig. 3). None of these trees supported a monophyletic *Cyprogenia aberti* due to the placement of two American Eastern Highland species, *C. stegaria* and *Dromus dromas*, within the *C. aberti* clades. In the Bayesian analysis, stationarity was achieved after 650,000 generations, and 8500 trees were discarded as burn-in. The 50% majority-rule consensus tree was based on the remaining 21,437 trees ($-\ln L = 4895.566$) and was similar to the MP topology (Fig. 4).

In both phylogenetic analyses, *Cyprogenia* form two major clades (A and B). Clades within the large A and B clades can be defined generally by river system membership of the *C. aberti* individuals (designated as Ouachita, White, or Arkansas) creating parallel geographic structure. Both clade A and clade B contain Ouachita (Ouachita [9], Saline [10], Caddo [11], sites, cf. Fig. 1) and White (St. Francis [3], Buffalo [4], Strawberry [5], Current [6], Black [7], White [8], sites, cf. Fig. 1) clades. Sympatric individuals from these two drainages segregate by conglutinate color: red conglutinate producers have clade A membership, while brown conglutinate producers belong to clade B (Fig. 2). Clade A also includes white conglutinate producers from the Fall River site (1, cf. Fig. 1) and the Verdigris River (see Serb, 2006) in the Arkansas Basin.

Only one node differs between the MP and Bayesian topologies. In the MP topology, the red conglutinate–producing Ouachita clade (Ouachita, Caddo, Saline exemplars) is the sister group to the White/*Cyprogenia stegaria* + Arkansas clade (2 decay index; less than 50% bootstrap support), while the Bayesian topology places the red conglutinate–producing Ouachita clade as the sister group to the White/*C. stegaria* clade (83 posterior probability). This topology places all red conglutinate producers together (Ouachita, White, and *C. stegaria* sites) and the white conglutinate producers in the Arkansas River as the basal group (Fall River site, Fig. 4).

Sequence divergence was calculated for each river drainage and phylogenetically defined groups within a single river (Table 1). Because CO1 and ND1 gene portions were determined by Modeltest to have two different sequence evolution models, we calculated sequence divergence for each gene separately. Evolutionary models for CO1 and ND1 were variations of the general time reversible (GTR) model: the Tamura-Nei model (TrN+Γ) with an adjustment for among-site rate heterogeneity ($\alpha = 0.2428$) for CO1, and the transversion model (TVM+I) with a parameter for the proportion of invariant sites ($I = 0.5211$) for ND1. Generally, sequence divergence within a phylogenetically defined clade was low, less than 1%. These groups corresponded to conglutinate color (red, brown, or white) within *Cyprogenia*. In contrast,

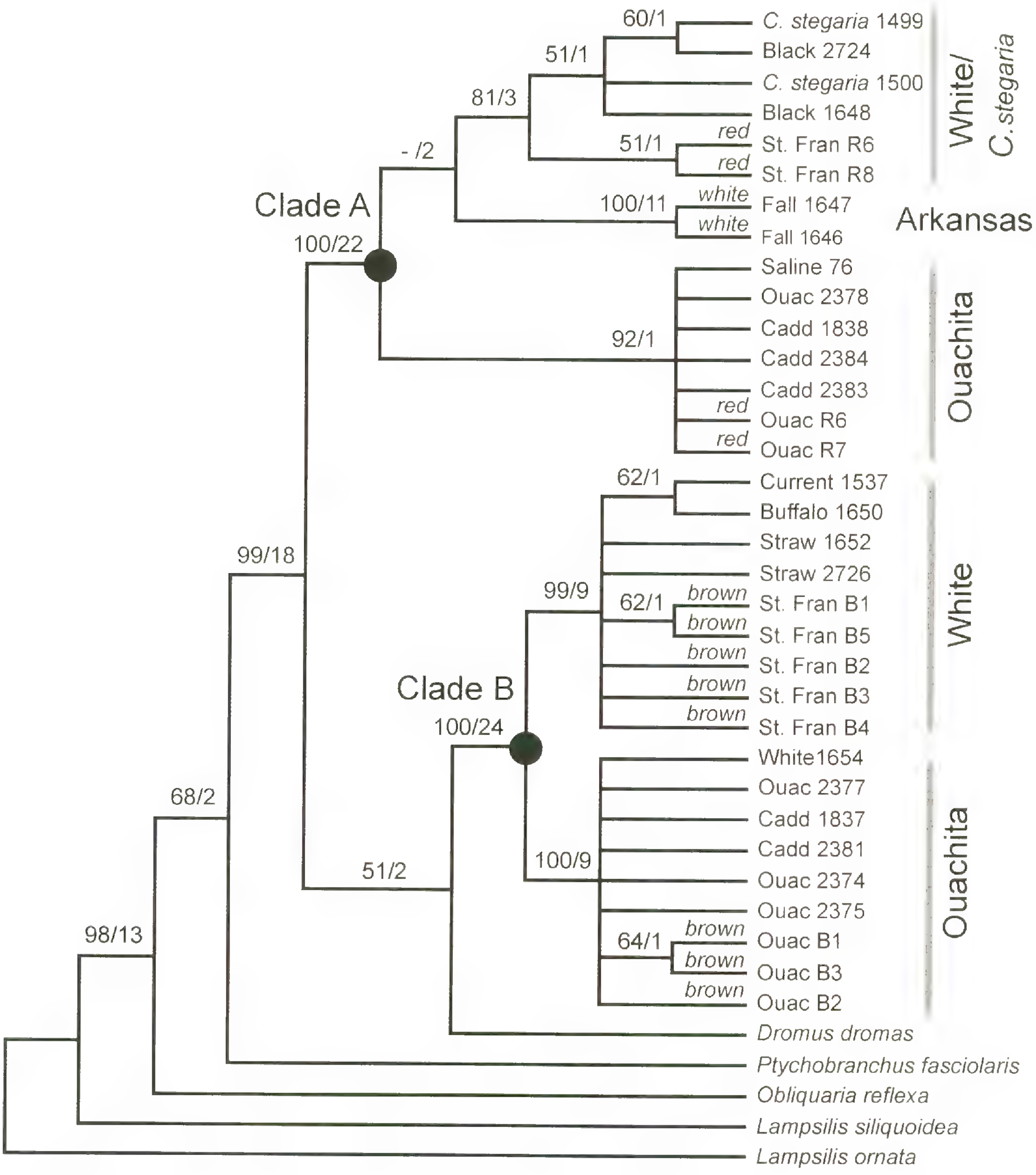

Figure 3. Strict consensus of 7476 trees recovered in maximum-parsimony analysis of the mtDNA sequence data set (tree length [TL] = 734; consistency index [CI] = 0.683; retention index [RI] = 0.895). Numbers above branches represent bootstrap support greater than 50% (2000 replicates) followed by decay index values. The two major *Cyprogenia* clades are labeled as A and B. Smaller clades are labeled by river drainage along terminal branches. Individuals with known conglutinates color are labeled (red, white, or brown) on terminal branches. Species currently recognized as *C. aberti* are listed by University of Alabama Unionid Collection museum number and river locality. River abbreviations are: Cadd = Caddo; St. Fran = St. Francis; Straw = Strawberry; Ouac = Ouachita. Other species are listed by species name in italics; *C. stegaria* specimens include museum accession numbers (see Appendix 1).

when means were calculated for drainages that included mitochondrially distinct, red and brown conglutinate producers, these values increased dramatically. Within a single location in the St. Francis River that contained red/brown conglutinate variation, individuals were more than 14% divergent (averages, CO1 = 14.6%; ND1 = 17.8%). These values were slightly higher in the Ouachita riverine system (averages, CO1 = 15.3%; ND1 = 20.2%). Again, these values are estimated from phylogenetically distinct genotypes (in A and B clades) found among sympatric individuals in two different river drainages

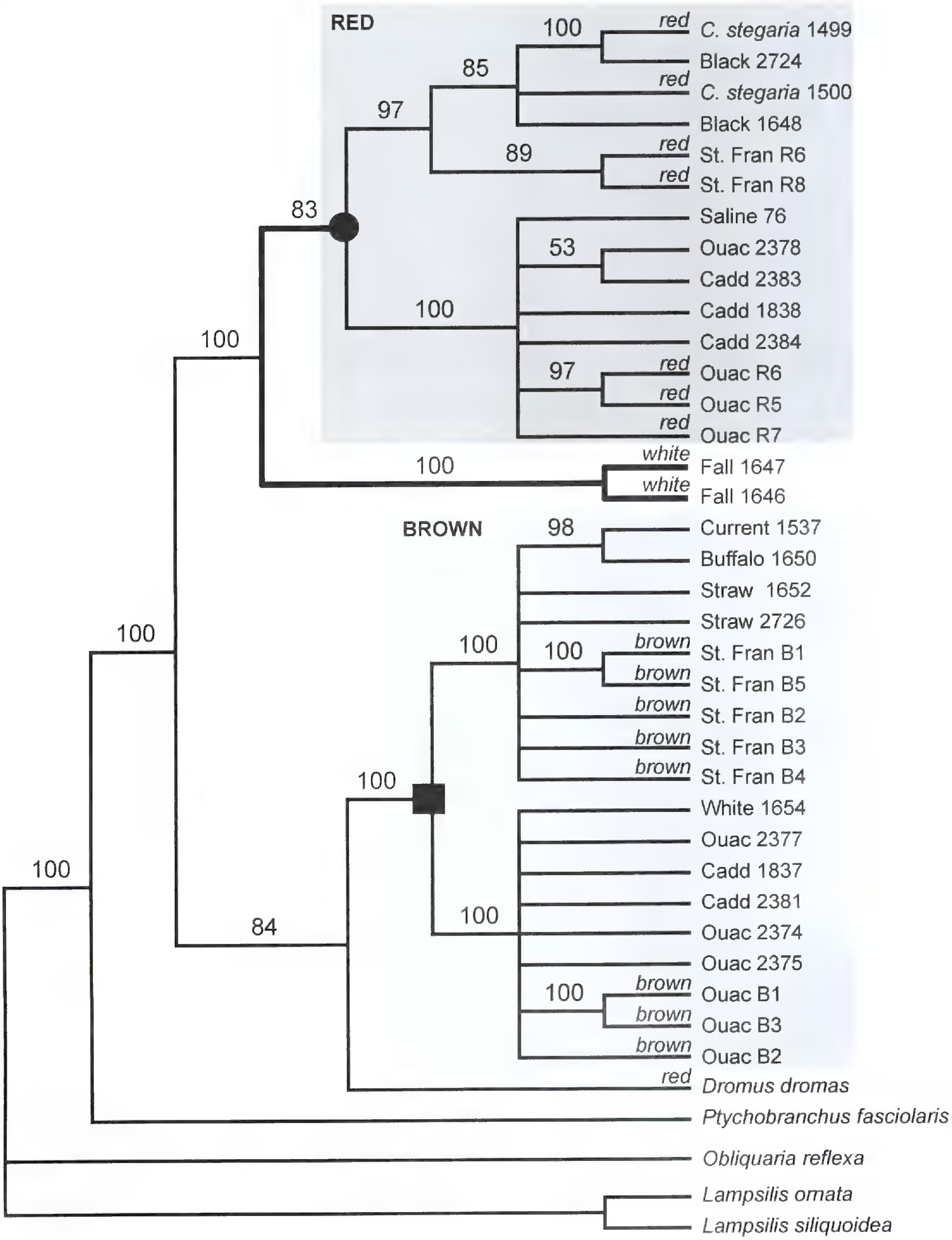

Figure 4. Bayesian 50% majority-rule consensus topology of the mtDNA sequence data set. Posterior probabilities based on 21,437 post burn-in trees (-ln L = 4895.566). Bold branches identify taxon relationships that differ from the MP analysis (Fig. 3). The clade containing individuals with red conglutinates is shaded in dark grey (node with circle). The brown conglutinate–producing clade is in light grey (node with square). *Cyprogenia aberti* from the Fall River (Arkansas drainage) produce white conglutinate lures. *Cyprogenia stegaria* and *Dromus dromas* are both red conglutinate producers (see references in text). Taxon labels follow Figure 3.

Table 1. Mean sequence divergence within drainage lineages (e.g., Arkansas), within conglutinate color lineages (e.g., red or brown), or between conglutinate color lineages (e.g., red vs. brown) as identified through phylogenetic analysis (see Fig. 3). Specific models of sequence evolution were determined for each mitochondrial gene portion: Tamura-Nei (TrN+Γ) corrected distances for the first subunit of cytochrome oxidase *c* gene (CO1); transversion (TVM+I) corrected distances for the first subunit of the NADH dehydrogenase gene (ND1).

	CO1	ND1
	TrN+Γ	TVM+I
White/*C. stegaria*	0.47%	0.54%
Arkansas	0.08%	0.82%
St. Francis		
Red	0.19%	0.35%
Brown	0.66%	0.21%
Red vs. brown	14.61%	17.76%
Ouachita		
Red	0.61%	0.47%
Brown	0.15%	0.09%
Red vs. brown	15.33%	20.19%

(Ouachita and St. Francis, within the White system) and not monophyletic entities.

Sequence alignment of the M- and F-mitotype copies of the CO1 gene yielded 529 bp, containing 253 polymorphic sites, 167 of which were phylogenetically informative under MP. The MP analyses resulted in four equally MPTs of 456 steps. None of the MP topologies placed the *Cyprogenia aberti* M-mitotype within the F-mitotype *Cyprogenia* clade (100% bootstrap support; Fig. 5). In addition, the dissimilarity between the amino acid sequence of the M- and F-mitotypes suggests that they are non-orthologous gene copies (data not shown; available from first author).

Discussion

Congruence between mitochondrial phylogeny and reproductive traits

This study attempted to test whether a mitochondrial gene tree with a replicated geographic pattern is an accurate assessment of the species tree in an organism with a complex life history. We compared the mitochondrial gene topology to life history trait patterns in *Cyprogenia aberti*. First, we examined a reproductive trait potentially key to the host-parasite association, conglutinate color. Variation in conglutinate lure color corresponded to the different mitochondrial lineages of *C. aberti*. All *Cyprogenia* produce worm-like conglutinates that are either white, red, or brown (Fig. 2). Each color morph appears to match clades defined by the mitochondrial analyses, thus supporting the mitochondrial gene phylogeny with an independent data set. Clade A has mostly red conglutinate producers, with the exception of white conglutinate producers of the upper Arkansas system (Fall River site). In contrast to clade A, clade B consists of brown-producing females, with the exception of *Dromus dromas*. Conglutinate lures produced by the closely allied *D. dromas* are morphologically distinct. On average, they are shorter, wider, and flatter than *Cyprogenia* conglutinates and, unlike *C. aberti* of clade B, are red in color when mature (Ortmann, 1912; Jones & Neves, 2004).

Other reproductive characters may be important in estimating biological diversity and understanding speciation mechanisms of *Cyprogenia aberti*. One trait is host fish preference. Although all *C. aberti* populations use small, benthic-dwelling species in the darter genera *Etheostoma* Rafinesque and *Percina* Halderman, host use varies among river basins (Barnhart, 1997; Eckert, 2003). Eckert (2003) found variation in host preference among four *C. aberti* populations: Spring (in Kansas) and Verdigris (2, Fig. 1) rivers of the Arkansas system, Ouachita River (9, Fig. 1), and St. Francis River (3, Fig. 1). All *C. aberti* populations examined had high transformation success (% of glochidia that complete development) on *P. caprodes* Rafinesque, a large darter species that is widely distributed, but not always locally abundant, across the range of *C. aberti* (Robison & Buchanan, 1992). In addition, each *C. aberti* population used at least one other host of more limited distribution. For example, *P. phoxocephala* Nelson, a host for Verdigris River *C. aberti*, co-occurs with *C. aberti* only in the upper Arkansas drainage (Page, 1983). Significantly, allopatric pairings of mussel populations and host species showed poor transformation success. However, differences in host compatibility between red and brown conglutinate–producing individuals were not evident (Barnhart, 1997; Eckert, 2003; Barnhart & Eckert, unpubl.). Difference in host compatibility among river drainages provides additional evidence for the distinction among genetically defined allopatric lineages of *C. aberti* and not the sympatric lineages in the Ouachita and White rivers. Change in host utilization may be an important factor in diversification of *C. aberti* lineages (see below).

Second, we looked for direct evidence of gene tree/species tree conflict by comparing two different mitochondrial lineages in *Cyprogenia aberti*. A copy of CO1 from the *C. aberti* M-mitotype was included in phylogenetic analyses to test the hypothesis that the

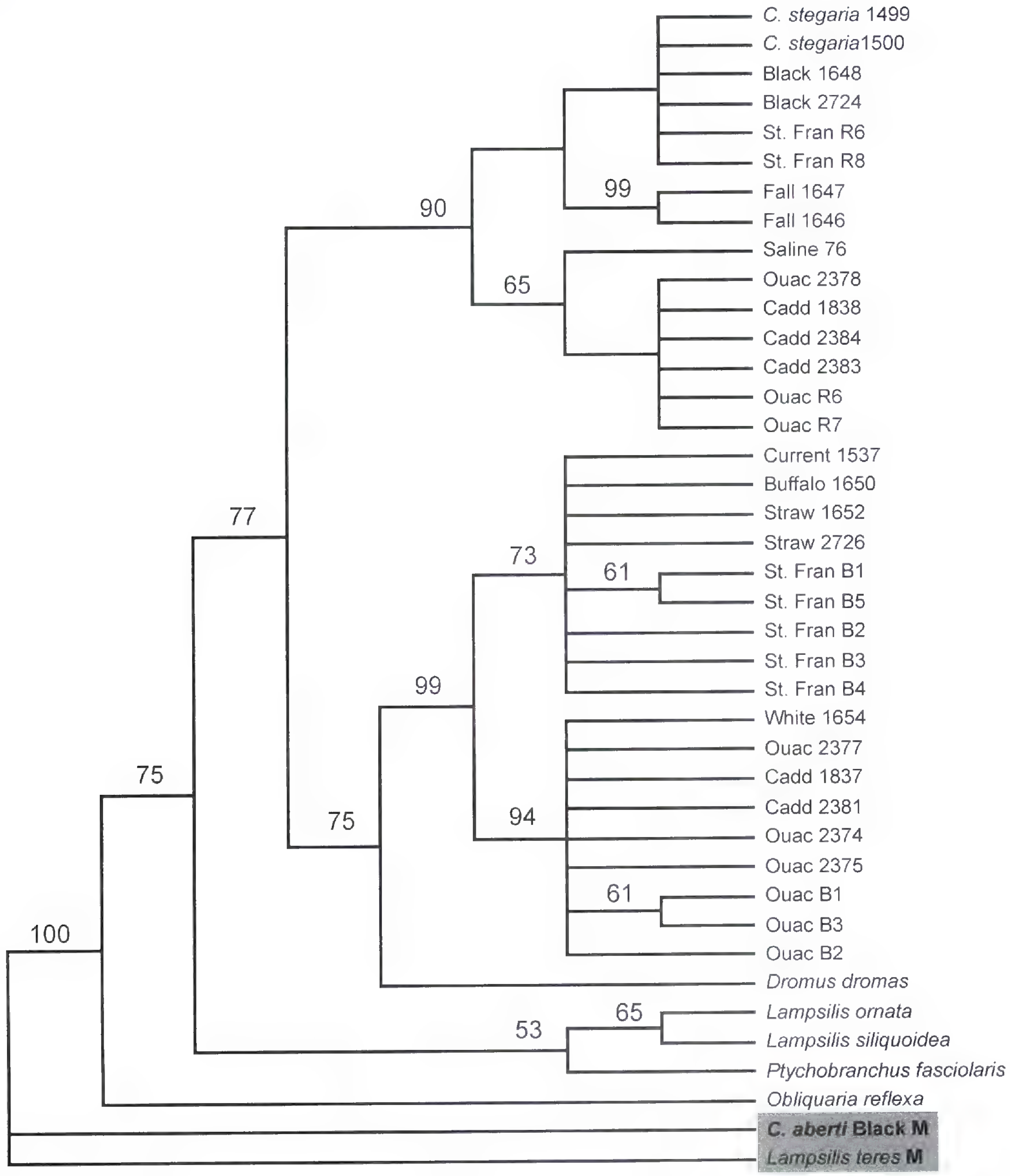

Figure 5. MP strict consensus topology comparing the male (M-) and female (F-) mitotype copies of CO1 (MPT = 4; TL = 456; CI = 0.724; RI = 0.871). Numbers above branches represent bootstrap support greater than 50% (2000 replicates). All individuals are F-mitotypes unless explicitly labeled as M-mitotypes. M-mitotypes are shaded in gray and have a bold 'M': *Cyprogenia aberti* Black M and *Lampsilis teres* M. Taxon labels follow Figure 3.

genetically disparate but sympatric individuals are the result of a role reversal of sexual mitotypes. In the MP analysis, the *C. aberti* M-mitotype was never included in the *Cyprogenia* clade (Fig. 5), suggesting that none of the *C. aberti* copies of the CO1 gene were male in origin. Since the *C. aberti* M-mitotype was not similar to any female-derived gene copies, it is most likely that the genetic variation and replicated geographic pattern found in *C. aberti* is not due to a role-reversal event. Instead, the observed genetic diversity found in *C. aberti* mitochondrial sequences arose within the *Cyprogenia* F-mitotype lineage. These data support recent evidence that DUI has been operating at a high level of fidelity in Unionoidea for over 100 million years (Curole & Kocher, 2002; Hoeh et al., 2002; Walker et al., 2006).

EXPLANATIONS FOR DIVERSITY PATTERNS

Examination of the mitochondrial sequence data in conjunction with observed phenotypic diversity within populations of *Cyprogenia aberti* suggests existence of multiple evolutionary entities (a species complex). In this section, we present several possible processes that may have produced this observed diversity. One mechanism is that the two lineages (A and B) formed in allopatry, and the river systems where they co-occur represent a secondary contact zone (clades A and B in Figs. 3, 4). This hypothesis depends on both vicariance events and subsequent range expansions that may have been driven by abiotic changes during the Pliocene or Pleistocene. As glacial advances during these epochs altered the ecology and geology of the Central Highlands region (see Mayden, 1987). these changes impacted distribution patterns of the biota. Fluctuations in climate, water levels, and reorganization of drainage patterns within the Highlands could isolate and displace populations of aquatic fauna, while creating opportunity for re-colonization and range expansion at a later time. The post-glacial secondary contact hypothesis has been used to explain similar genetic patterns in other molluscan taxa, including slugs (Pinceel et al., 2005) and terrestrial gastropods (Davidson, 2000).

Sympatric speciation is another process that can generate genetically diverse sympatric lineages. It has been demonstrated that shifts in reproductive timing can result in sympatric speciation (allochrony) in invertebrate taxa, specifically those that have complex mating behaviors or periodicity, or those that utilize hosts (Wood & Guttman, 1982; Feder et al., 1993; Harrison & Bogdanowicz, 1995; Simon et al., 2000). *Cyprogenia aberti* possess a complex life cycle that provides many opportunities for allochronic speciation, where a shift in the timing of early reproductive events (i.e., gamete release, brooding, or larval release) could affect the success of their obligate parasitic larva. For example, larvae from animals that reproduce later in the reproductive season may be exposed to a different suite of potential fish hosts (Graf, 1997). If allochrony is a speciation mechanism in *C. aberti*, it does not appear to be a recent event, as evident from the non-sister clade pattern of the Ouachita groups. Instead, allochrony may have occurred within the Ouachita system, followed by range expansion of the two new lineages into other Highland drainages. Additional life history, geological, and perhaps nuclear gene sequences and molecular clock data will be needed to determine which speciation mechanism created the diversity in *C. aberti*.

Regardless of how the sympatric lineages in the Ouachita and White river systems were formed, the question remains of how the color polymorphism could be maintained evolutionarily. Either the maintenance of color polymorphism within one species or the coexistence of sympatric species could result from frequency-dependent selection. In this model, host fish might avoid conglutinates after an initial encounter because of discomfort associated with glochidia attachment. Mussels producing a rare conglutinate color might, therefore, experience a selective advantage because fish would be less likely to have previous experience with that color. The selective advantage of a given color would increase with decreasing frequency in the population, protecting the polymorphism. It is necessary to establish several phenomena in order for this theory to gain credence, including the aversion of fishes to similar-colored conglutinates after an initial exposure. This theory also does not explain the very large genetic differences observed in the mitochondrial sequence data.

CONCLUSIONS

Gene trees are a critical first step when assessing biological diversity, but gene trees may be suspect if organisms have complex traits, such as unusual modes of genetic inheritance. We advocate that a comparative approach is the best method to assess biological diversity in these under-studied taxa. In general, we found congruence among the different data sets for *Cyprogenia aberti*. The congruence of conglutinate color and the mitochondrial gene tree appears to support separate nuclear lineages in *C. aberti* without direct evidence from a nuclear marker. In invertebrates, color morphs associated with mitochondrial lineages are not uncommon, and these data have been used to identify species even when color forms are otherwise morphologically and ecologically indistinguishable (Tarjuelo et al., 2004). If this precedent is followed, up to five species could be described in the *C. aberti* complex. Within the Ouachita River, two sympatric species exist: the red conglutinate producers (cf. Fig. 3, clade A) and the brown conglutinate producers (cf. Fig. 3, clade B). A similar division occurs in the White River, with red conglutinate producers and brown conglutinate producers co-occurring in the St. Francis River (Fig. 3). Future biological surveys of rivers in the White River system will be needed to determine these two species' ranges. Lastly, the upper Arkansas River contains a single species that produces white conglutinates.

In contrast to conglutinate color, the data on host fish indicate that there are no differences in host utilization among sympatric red and brown conglutinate forms within the Ouachita and White systems.

These data do not support, but also do not falsify, the hypothesis of sympatric species. Both genetic differences and host compatibility differences among geographic populations are consistent with limited gene flow and the possibility of several species within the *Cyprogenia aberti* group. These geographically separated *C. aberti* clades are not ecologically exchangeable. This conclusion is based on genetic divergence among lineages, difference in reproductive traits, such as conglutinate morphology, and preliminary data on host fish preference. Finally, the corroboration among different data sets suggests that *C. aberti* is a species complex that contains cryptic biological diversity. This diversity will be formally described in a forthcoming manuscript.

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Appendix 1. Voucher information for specimens sequenced in this study. GenBank accession numbers for the first subunit of cytochrome oxidase *c* (CO1) and the first subunit of the NADH dehydrogenase (ND1) genes, respectively, are in parentheses. Voucher specimens are deposited at the University of Alabama Unionid Collection (UAUC) and are listed by museum accession numbers. Locality information and GenBank accession numbers for other species are found in Serb (2006).

Cyprogenia aberti:

Fall River (1). U.S.A. Kansas: Wilson Co., University of Alabama Unionid Collection [UAUC] 1646 (DQ494735, DQ494711), UAUC 1647 (DQ494728, DQ494708).

St. Francis River (3). U.S.A. Missouri: Wayne Co., UTM 15 722800E 4123900N (Patterson quad), B1 (DQ667060, DQ663617), B2 (DQ667061, DQ663618), B3 (DQ667062, DQ663619), B4 (DQ667063, DQ663620), B5 (DQ667064, DQ663621), R6 (DQ667065, DQ663622), R8 (DQ667066, DQ663623).

Buffalo River (4). U.S.A. Arkansas: Newton Co., UAUC 1650 (DQ494730, DQ494710).

Strawberry River (5). U.S.A. Arkansas: Sharp Co., UAUC 1652 (DQ494744, DQ494719), UAUC 2726 (DQ494745, DQ494720).

Current River (6). U.S.A. Arkansas: Clay Co., UAUC 1537 (DQ494727, DQ494707).

Black River (7). U.S.A. Arkansas: Randolph/Lawrence Co., UAUC 1648 (DQ494729, DQ494709), UAUC 2721 (DQ494742, DQ494717). Missouri: Butler Co., UTM 15 732729E 4071085N, M-mitotype (DQ663624).

White River (8). U.S.A. Arkansas: Woodruff/Jackson/White Co., UAUC 1654 (DQ494736, DQ494712).

Ouachita River (9). U.S.A. Arkansas: Montgomery Co., UTM 15 434359E 3828410N (Mount Ida quad), B1 (DQ667054, DQ663611), B2 (DQ667055, DQ663612), B3 (DQ667056, DQ663613), R5 (DQ667057, DQ663614), R6 (DQ667058, DQ663615), R7 (DQ667059, DQ663616).

Saline River (10). U.S.A. Arkansas: Saline Co., UAUC 76 (DQ494733, AY158749).

Caddo River (11). U.S.A. Arkansas: Pike Co., UAUC 1837 (DQ494739, DQ494715), UAUC 1838 (DQ494743, DQ494718). Arkansas: Clark Co., UAUC 2381 (DQ494741, DQ494716), UAUC 2383 (DQ494749, DQ494724), UAUC 2384 (DQ49447, DQ494722).

Cyprogenia stegaria:

Clinch River. U.S.A. Tennessee: Hancock Co., UAUC 1499 (AY654992, AY655089), UAUC 1500 (DQ494726, DQ494706).

THE IMPACT OF PETER RAVEN ON EVOLUTIONARY AND BIODIVERSITY ISSUES IN THE 20TH AND 21ST CENTURIES: INTRODUCTION[1, 2]

Peter C. Hoch[3]

The 53rd Annual Systematics Symposium of the Missouri Botanical Garden, held on 13–14 October 2006, had as its theme "The Impact of Peter Raven on Evolutionary and Biodiversity Issues in the 20th and 21st Centuries." This focus on the work and impact of one individual, a departure from previous Garden symposia based on single topics, was occasioned by the celebration in 2006 of the 35th anniversary of Peter Raven's arrival as Director of the Missouri Botanical Garden. Given that two thirds of the Garden's annual symposia have taken place during his tenure, this seemed a particularly appropriate opportunity to celebrate Peter Raven's intellectual contributions to the broader scientific community and, indeed, to the world at large.

A committee of Raven's colleagues (Olga Martha Montiel, Barbara Schaal) and former students (Paul Berry, Peter Hoch, and Warren L. Wagner) convened to decide what issues to cover and who would speak. The selection process was difficult, given the breadth of Raven's intellectual contributions and the constraint of only eight speaking slots. Faced with his massive publication record (more than 650 entries at the end of 2005), the committee chose topics to which Raven made major contributions that continue to be important areas of research, hence the selection of population biology/gene flow, biogeography, coevolution, pollination biology, and cytogenetics. Other areas to which Raven has made immense contributions are less easily characterized, but we determined that the broad area of conservation/biodiversity certainly should be included. Finally, in thinking not only about the exceptional intellectual leadership he has provided to the Garden for 35 years, to his own students, and to legions of other students influenced by his three top-selling college textbooks, but also about the many institutions he has served with distinction—the National Academy of Sciences, the National Geographic Society, the National Science Board, among others, the universities at which he has held positions, and the many scientific societies to which he belongs or has been elected—we decided we needed a talk that would address Raven's contributions to "building capacity" in the broadest sense. Fortunately, to round out the final slate of eight talks, Paul Ehrlich agreed to give the evening address, which provided a more personal perspective on the very fruitful collaboration he shared with Raven over the course of more than 40 years.

Due to various extenuating circumstances, not least the unique historical perspective adopted by most of the speakers, only two manuscripts resulted from this symposium, those by Diane Campbell (pollination biology) and J. Chris Pires and Kate L. Hertweck (cytogenetics), which follow this introduction. What follows here is a brief synopsis of the remaining talks, in the order of their presentation at the symposium.

Barbara Schaal (Washington University) presented "Differentiation of Populations: Gene Flow Redux," revisiting the seminal paper published in *Science* in 1969 (Ehrlich & Raven, 1969), "Differentiation of Populations." This paper challenged the prevailing orthodoxy that species were evolutionary units held together by gene flow, and opened a broad and productive field of research into the empirical

[1] This and the two articles that follow it are the proceedings of the 53rd Annual Systematics Symposium of the Missouri Botanical Garden, "The Impact of Peter Raven on Evolutionary and Biodiversity Issues in the 20th and 21st Centuries." The symposium was held 13–14 October 2006, at the Missouri Botanical Garden in St. Louis, Missouri, U.S.A.

[2] This was the 51st Missouri Botanical Garden Annual Systematics Symposium to be supported by a grant from the National Science Foundation (grant DEB-0515933). The meeting was organized by Barbara Schaal of Washington University and Peter Hoch of the Missouri Botanical Garden, with the assistance of Paul Berry (then at University of Wisconsin) and Warren Wagner (Smithsonian Institution). P. Mick Richardson was responsible for the smooth running of the symposium, with the welcome and able assistance of Mary McNamara, Sandy Lopez, Donna Rodgers, William Guy, and Zubin Chandran. Victoria C. Hollowell, Beth Parada, and Allison Brock (Missouri Botanical Garden Press) were responsible for the publication of the symposium proceedings. This symposium, together with 36 previous ones, again benefited considerably from the contributions, support, and encouragement of Peter Raven.

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measure of gene flow, which remains active and vibrant to the present. Through the use of several model plant systems, Schaal explored the processes that enhance and retard population differentiation.

Stephen J. O'Brien (Laboratory of Genomic Diversity, National Cancer Institute) presented "Genomic Archaeology—Solving the Mysteries of History," which explored the topic of diversity from the perspective of comparative genomics in mammals, and reported recent advances in conservation genetics. Using examples from his remarkable genomic research on such diverse mammals as cats and elephants (O'Brien, 2003), O'Brien discussed new approaches to the elucidation of biological diversity, with applications for sampling methods and the analysis of extinction, in these topics echoing many publications by Raven (e.g., Phillips & Raven, 1997; Raven & McNeely, 1998).

Ulrich Mueller (University of Texas at Austin) presented "Coevolutionary Principles of Insect Fungiculture: Lessons for Human Agriculture?," which demonstrated the rich intellectual fruit borne from the original planting of the idea of coevolution by Ehrlich and Raven (1964) in their article in *Evolution*, "Butterflies and Plants: A Study in Coevolution." Using the remarkable adaptations found in fungiculturist attine ants (Gerardo et al., 2006), Mueller demonstrated the sophistication of recent coevolutionary analyses and presented the ant-fungus-microbe interactions as an intriguing analogy to human agriculture, with important potential applications for improving the latter.

Jun Wen (National Museum of Natural History, Smithsonian Institution) presented "Evolution of Major Patterns of Plant Disjunctions," reviewing and updating many of the hypotheses proposed by Raven over many years (e.g., Raven, 1963), most notably in his collaborative work with the late Daniel Axelrod (e.g., Raven & Axelrod, 1974, 1978). Wen enumerated the major intercontinental disjunction patterns, with many examples from recent work, noting the insights gained from modern phylogenetic analyses and the nascent field of lineage dating based on fossil and molecular data.

Cristián Samper (National Museum of Natural History, Smithsonian Institution) presented "A Raven's Garden: Building Capacity for Plant Research and Conservation," in which he discussed the need for building capacity in all countries and how that relates to the rapid destruction of biological diversity worldwide. He then explored some of the impacts that Peter Raven has had in this area, developing flora projects in many biodiverse regions, placing staff in the countries of origin, developing tools to enhance the flow of information about species, and developing long-term partnerships for conservation.

Paul Ehrlich (Stanford University) closed the Symposium with his evening address, "Saving the World," an informal and humorous recollection of his long and fruitful collaboration with Raven, and how some of those ideas have developed into new fields of study. With a playful allusion to the title of one of Raven's (1988: "We're Killing Our World") singular contributions on conservation and the human impact on the global ecosystem, Ehrlich shared his deep passion for the study of biology and the need for personal responsibility in conservation (Ehrlich, 2000), and his personal insights and humor regarding his relationship with Peter Raven made for a fitting close to a day of celebration.

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POLLINATOR SHIFTS AND THE ORIGIN AND LOSS OF PLANT SPECIES[1]

Diane R. Campbell[2]

Abstract

Pollinators have long been implicated in plant speciation. Peter Raven's earlier work was instrumental in integrating foraging energetics of animals into our understanding of how shifts in major pollinators influence the evolutionary diversification of floral traits. More recently, efforts by Raven and others in the area of conservation have inspired pollination biologists to consider the implications of pollinator shifts and losses due to human activities. This paper uses the shift between hummingbird and hawkmoth pollination as a model for exploring impacts of pollinator shifts on plant populations. Recent studies have quantified the degree of reproductive isolation due to such pollinators in several genera. Data from *Ipomopsis* Michx. further allow us to consider whether recent changes in pollinator regimes have demographic consequences for plant populations. A majority of plant populations may currently suffer from pollen limitations on seed production, but few data exist on the demographic consequences of poor reproduction. In *Ipomopsis*, reduced seed production due to pollen limitation can impact the number of individuals surviving to reproduce in the next generation. Some populations of *I. tenuituba* (Rydb.) V. E. Grant are estimated to have finite rates of increase less than unity, which can be explained in part by current low levels of hawkmoth pollination. In the absence of an increase in hawkmoths, selection for wider corolla tubes and other floral traits could, in principle, attract enough hummingbird pollination to result in a growing population, but models show that such evolution by natural selection may leave the population vulnerable to local extinction. We need more studies of the quantitative demographic consequences of changes in pollinator regimes. Such studies should consider how evolutionary changes influence the risk of extinction.

Key words: *Aquilegia*, coevolution, extinction, hawkmoth, hummingbird, *Ipomopsis*, *Nicotiana*, pollen limitation, pollinator shift, speciation.

Changes in pollinator regimes are thought to be of critical importance in speciation and diversification of the flowering plants. Such changes include qualitative shifts from one type of pollinator to another, quantitative shifts in the relative proportions of major types, and overall reductions in visits by animal pollinators that select for wind pollination or self-fertilization. In the past few decades, biologists have made considerable progress in testing the role of pollinator shifts in fundamental aspects of the origin of species: diversification of floral traits and development of reproductive barriers between incipient species. They have also begun to evaluate the role played by recent changes in pollinator regime for the potential loss of species. One major goal of this paper is to explore the roles played by pollinator changes in both the origin and the loss of species, using hummingbirds and hawkmoths as a case study and paying attention to the historical roots of these ideas, particularly those resulting from the work of Peter Raven and his colleagues. A second major goal is to integrate the two themes by considering how we can incorporate evolutionary studies into examining the impact of pollinator losses. Where data are otherwise scarce in the current literature, I will focus on studies of *Ipomopsis* Michx. (Polemoniaceae), including ongoing work.

Peter Raven and Pollination Biology

Pollination is central to so much of angiosperm evolution that it perhaps should not surprise us that so many diverse aspects of Raven's work have made a mark on pollination biology. Although Raven's experimental work in pollination focused mainly on specialized pollination systems in the Onagraceae, perhaps his widest impact on this field can be traced to three papers that introduced new conceptual approaches during the 1960s and 1970s. Prior to that time, much of pollination biology emphasized descriptive analysis of associations between flower characters and types of pollinators (Faegri & van der Pijl, 1966). One of the biggest changes in pollination biology occurred with a fuller incorpora-

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tion of the animal's perspective, a change precipitated, in part, by publication of Ehrlich and Raven's (1964) landmark paper on coevolution and Heinrich and Raven's (1972) fundamental paper on foraging energetics of pollinators. In addition, the idea that gene flow is too restricted to be responsible for holding together a species (Ehrlich & Raven, 1969) sparked a whole industry focused on estimating movement of pollen (e.g., Levin & Kerster, 1974). These three papers by Raven helped to launch a renaissance in pollination biology, as biologists increasingly recognized plant-pollinator systems as models for understanding major issues in ecological and evolutionary processes. As the ideas of gene flow through pollen, and of coevolution, are considered elsewhere in the symposium, I will focus on what follows on other influences, especially the perspective brought by considering animal foraging.

Pollinator Shifts, Floral Diversification, and Origin of Plant Species

Hummingbird versus insect pollination

Some of the most compelling evidence for the role of pollinators in speciation comes from studies of related plant species that are pollinated either by hummingbirds or by insects. This emphasis is perhaps natural, given the longstanding interest in features often found in hummingbird-pollinated plants: red, tubular flowers that are relatively broad (compared, for example, to moth-pollinated flowers) and produce large quantities of relatively dilute nectar (compared to bee-pollinated flowers; Bolten & Feinsinger, 1978). Raven (1972) considered the question of why so many plants visited by hummingbirds have red flowers. He argued that birds learn to recognize red as a signal for a large caloric nectar reward even though they have no intrinsic preference for red, and that red is the signal because it is less conspicuous to most insects. This topic has continued to fascinate pollination ecologists and remains not fully resolved. Recent studies have altered the picture by demonstrating that bees can perceive red flowers, although the color is more difficult for them to distinguish against a green background of leafy vegetation (Chittka & Waser, 1997); this results in increased handling time and presumably renders red flowers less efficient, but not invisible, for bee foraging (Spaethe et al., 2001). The primary significance of reward energetics present in Raven's original argument for flower choice has been amply documented and reinforced. This emphasis on rewards was fleshed out in the highly influential paper by Heinrich and Raven (1972). The idea, simply put, was that the caloric reward of a flower could be used to predict which pollinator species would visit it, the number of flowers visited per plant, and other aspects of foraging biology that could influence important features of plant reproduction such as the outcrossing rate. Along with the concept of coevolution between insects and plants elaborated by Ehrlich and Raven (1964), this paper stimulated a new generation to work on pollination from both the plant and animal points of view. In particular, it emphasized the idea that one could predict aspects of pollination by understanding the foraging energetics of the animals (Waddington, 1983), an idea that also owed much to the interjection of foraging theory (Pyke, 1984). Foraging energetics ultimately offered new perspectives on the adaptive significance of many plant traits, such as number of flowers per inflorescence (Wyatt, 1982), nectar distribution across flowers (Best & Bierzychudek, 1982), nectar guides (Waser & Price, 1983), and packaging of pollen (Harder & Wilson, 1994).

Quantifying pollinator-mediated selection and reproductive isolation

These ideas about foraging energetics also revitalized work on speciation resulting from pollinator shifts. Speciation involves divergence of phenotype and development of reproductive isolation, and both could be produced by pollinator shifts. Different types of pollinators (for example, bumblebees vs. birds) have different energetic requirements, morphologies, and sensory systems that may lead them to visit plants with different suites of floral traits. Such behavioral differences would produce divergent selection mediated by pollinators, potentially leading to differentiation of the floral phenotype, and could also generate reproductive isolation (see below). While this viewpoint was clearly articulated early on (Grant, 1949), it took much longer for biologists to document selection mediated by pollinators. In an introduction to an influential volume on pollination biology published two decades ago (Real, 1983), Raven remarked that it will ultimately be necessary to document that genetically variable floral traits actually contribute to reproductive success. This feat has since been accomplished for several species (e.g., *Raphanus raphanistrum* L. by Stanton et al., 1986; *Polemonium viscosum* Nutt. by Galen, 1996); however, most studies have focused on selection within a particular plant species. Only recently has the question of divergent selection and reproductive isolation by divergent pollinators been addressed with the same rigor. However, recent studies of genera with both hummingbird and hawkmoth pollination (*Aquilegia* L., *Ipomopsis*, and *Nicotiana* L.) offer repeated examples in which the extent of reproductive isolation mediated by pollinators has been quantified.

To see how reproductive isolation has been quantified, it is important to consider in more detail how pollinator behavior can generate it. With pollinator-mediated divergent selection, hybrids between the two plant species could have low pollination and, therefore, low fitness, a form of postzygotic reproductive isolation. Furthermore, if pollinators focused on a single species because of its more suitable reward or morphology, pollen movement between species would be restricted, causing prezygotic reproductive isolation as well. This contribution of behavior to reproductive isolation is referred to as ethological isolation ($RI_{ethological}$) and can be quantified as:

$$RI_{ethological} = 1 - \frac{heterospecific\ plant\ transitions}{conspecific\ plant\ transitions}$$

where transitions refer to movements made by pollinators in a setup with equal choices available. For cases of species visited predominantly by hummingbirds and hawkmoths, estimates for ethological isolation range from 0.50–1.00, a considerable range (Campbell & Aldridge, 2006) that begs further consideration. A second form of reproductive isolation (mechanical isolation) can also be produced by pollinators when they move pollen more efficiently between conspecific flowers because of differences in aspects of floral morphology such as the positions of reproductive organs.

CASE STUDIES WITH HUMMINGBIRDS AND HAWKMOTHS

Two species of *Aquilegia* illustrate the case of strong ethological isolation and show the contribution of at least one specific floral trait (Fulton & Hodges, 1999). *Aquilegia formosa* Fisch. has pendent, red and yellow flowers with short nectar spurs, whereas *A. pubescens* Coville has upright, pale flowers with long spurs. Although visited primarily by hummingbirds and hawkmoths, respectively, the two species produce hybrid zones in the southern Sierra Nevada of California, a situation originally studied by Chase and Raven (1975). In an array of *A. pubescens* in which half of the flowers were tied to make the flowers pendent and, thus, given the trait of the other parental species, hawkmoths visited upright flowers more than 10 times as often as pendent flowers (Fulton & Hodges, 1999). Manipulation of this one trait produced strong preference for *A. pubescens*; however, other floral traits such as flower color are likely also involved, as the preference was still not as extreme as observed in arrays of the two parental species (Fulton & Hodges, 1999). Although flower orientation is clearly involved in strengthening the current reproductive isolation, it is not clear whether this trait was also involved in the original speciation event, particularly without information on how each trait influences the energetics of nectar extraction by hawkmoths. Hodges and his collaborators are now studying the genetic basis of the floral traits that maintain reproductive isolation (Hodges et al., 2002).

Nicotiana alata Link & Otto and *N. forgetiana* Hort. ex Hemsley in Brazil provide a similar example in which prezygotic reproductive isolation is normally strong (Ippolito et al., 2004). In experimental plots with only plants of the two species, hummingbirds visited *N. forgetiana* exclusively, and hawkmoths strongly preferred *N. alata*, in agreement with their pollinator syndromes. However, the presence of some hybrids led to high interspecific pollen transfer, producing a "hybrid bridge" between the species (Leebens-Mack & Milligan, 1998). When plots contained the two species along with F_1 individuals, hummingbirds visited both species, so that their movements could have produced interspecific gene flow, and backcrossing between hybrids and the parental species was also possible. This finding supports the suggestion of Arnold (1997) that even if F_1 hybrids rarely arise due to prezygotic ethological isolation, their presence can accelerate further gene flow between species.

RELATIVE IMPORTANCE OF SELECTION MEDIATED BY POLLINATORS AND HABITATS

Besides pollinator differences, an alternative form of selection that could drive plant speciation is differences in habitat such as soil types, other physical attributes of the environment, or biotic interactions that are unrelated to pollinators (Waser & Campbell, 2004). Adaptation to different habitats could generate postzygotic reproductive isolation if hybrids are unfit as a result of genomic incompatibilities or specifically unfit in the parental environments (Hatfield & Schluter, 1999). Chase and Raven (1975) proposed that habitat differences might actually be more important than pollinator differences in maintaining the species difference between *Aquilegia*. In principle, these two forms of selection could be distinguished by comparing the extent to which hybrids suffer from low pollination success versus low survival or other aspects of fitness. Few studies have examined pollination success of plant hybrids, although visitation to hybrids was studied in the *Nicotiana* arrays mentioned above (Ippolito et al., 2004).

In *Ipomopsis* (Polemoniaceae), the importance of both pollinator-mediated and habitat-mediated selection has been investigated. As in the two cases mentioned above, the two species involved differ conspicuously in floral traits. *Ipomopsis aggregata*

(Pursh) V. E. Grant has broad-tubed red flowers with high nectar production and often exserted anthers and stigma, whereas *I. tenuituba* has narrow-tubed, long, white or pale pink flowers with lower nectar production and strongly inserted reproductive parts. These two species are close relatives (Wolf et al., 1993), and Grant and Grant (1965) suggested they provide an example of recent or incipient speciation via pollinator-mediated divergent selection in which *I. aggregata* is pollinated by hummingbirds and *I. tenuituba* by hawkmoths. However, at some contact sites, the two species are visited by both pollinator types when present, and, as a result, they often form extensive hybrid zones in nature (Grant & Wilken, 1988; Wu & Campbell, 2005).

One such hybrid zone occurs in Poverty Gulch in Gunnison County, Colorado. Hybrids between the two species of *Ipomopsis* taken from that area are easily made, with just as many seeds resulting from heterospecific crosses as from conspecific crosses (Campbell & Waser, 2001). To determine how hybrids compare with the parental species in pollination success versus other aspects of fitness, Campbell and Waser (2007) planted more than 3000 seeds from conspecific and heterospecific crosses (F_1 reciprocal hybrids) into the two parental sites and a site where natural hybrids are common and followed the progeny for 12 years until nearly all of these monocarpic perennial plants had completed their life cycle. Differences in survival and reproduction both made substantial contributions to the fitness differences. Overall, survival of F_1 hybrids was equivalent to the average of the parental species; however, in the two parental sites, the direction of the cross was critical. Hybrids with *I. tenuituba* as the mother survived well only in the natural hybrid site, suggesting that habitat differences are important. One factor involved is water availability. The hybrid site is warmer and drier than the parental sites, and the hybrids have higher photosynthetic water-use efficiency than the parental species (Campbell et al., 2005). Pollination is also critical. In experimental arrays of potted plants with equal numbers of each of the parental species and hybrids, plants of *I. aggregata* received and donated the most pollen to stigmas of other plants (as estimated by dyes), thereby enjoying the overall highest pollination success (Campbell et al., 2002). Hybrid pollination success was intermediate between that of the two parental species in one experiment and equal to *I. aggregata* in another. In a separate study, hybrids produced and sired intermediate numbers of seeds to those of the parental species (Meléndez-Ackerman & Campbell, 1998). So, hybrids do not suffer low pollination success in situations where only hummingbirds are present.

These patterns in pollination success reflect primarily the visitation preference of hummingbirds for *Ipomopsis aggregata* and hybrids rather than *I. tenuituba* (Campbell et al., 1997). In this area, two species of hummingbirds, broad-tailed hummingbirds and rufous hummingbirds, make most of the flower visits. Their preference also results in partial ethological isolation, with about 7% as much pollen transferred between species as transferred conspecifically (Campbell et al., 2002). Mechanical isolation contributes as well, although to a lesser extent (Campbell et al., 1998).

The visitation preference of hummingbirds reflects the wider corolla tubes, red color, and greater nectar production of *Ipomopsis aggregata* (Campbell et al., 1997; Meléndez-Ackerman & Campbell, 1998). In particular, the preference of hummingbirds for *I. aggregata* generates directional selection for wide corolla tubes. One species of hawkmoth (*Hyles lineata*) is also a visitor at this study site but only in rare years (two years out of the last 20). In years when they are present, they disproportionately visit plants with narrower tubes. The combination of birds and moths in the natural hybrid zone then generates disruptive selection on corolla width, in line with the expectation that pollinator-mediated divergent selection maintains species differences (Campbell et al., 1997).

Campbell and Waser (2007) used life table analysis to show that differences in age-specific survival and fecundity both made large contributions to the patterns of lifetime fitness seen in the plants in the reciprocal transplant study described above. The low fitness of *Ipomopsis tenuituba* at *I. aggregata* sites could be explained by low pollination, whereas the low fitness of F_1 hybrids with *I. tenuituba* mothers at that site reflected poor survival. In summary, both pollinator-mediated and habitat-mediated selection contribute to the fitness differences seen in this hybrid zone and to maintenance of species differences. They may also have contributed to the original speciation between these sister species (Wolf et al., 1993).

Another way to approach the problem of the role played by pollinator-mediated selection is to compare cases where species hybridize with cases where they do not, to see if the lack of hybridization can be explained by lack of shared pollinators. Such a comparison is most informative if it involves the same species pair in different localities. *Ipomopsis aggregata* and *I. tenuituba* show considerable geographical variation in the extent of hybridization. At Grizzly Ridge on the north rim of the Black Canyon of Gunnison, Colorado, there are few if any morphological hybrids. That site thus provides an extreme contrast with the Poverty Gulch site, where hybridization is extensive. This difference can be explained

by differences in behavior of pollinators between the two sites. At Grizzly Ridge, the same species of hummingbirds and hawkmoths found at Poverty Gulch are completely species-specific in foraging. Experiments with potted plants in four combinations, representing all combinations of site of origin of the plants and site of observation, showed that the difference in ethological isolation was primarily the result of hawkmoths changing their behavior between sites (Aldridge & Campbell, 2007).

In summary, the extent of reproductive isolation due to behavior of pollinators has now been documented in at least a few cases, with *Aquilegia*, *Nicotiana*, and *Ipomopsis* serving as examples of species pairs involving hummingbird and hawkmoth pollination. More work will be needed, however, to quantify the general importance of these differences in pollinators versus differences in habitats, to the two facets of plant speciation: divergence in phenotype and development of reproductive isolation.

Anthropogenic Changes in Pollinator Regimes and Potential for Species Loss

potential consequences

Plants and their pollinators, like other parts of the natural world, face increasing peril from human activities. One example is the recently documented parallel decline in insect pollinators and insect-pollinated plants in Britain and The Netherlands (Biesmeijer et al., 2006). Understanding and mitigating these perils is an important challenge for pollination ecologists and an important arena for future work. Peter Raven's extensive work in conservation biology has helped to fuel wide concern over threats such as habitat loss and fragmentation, global warming, and invasive species (Raven, 2002). While a recent report from the National Academy of Sciences (National Research Council, 2007) points out the considerable gaps in our knowledge of effects on pollination, concern over the potential for harm is rising. Potential consequences of pollinator changes extend not only to native plant populations but also to agriculture, as 66% of the world's crops depend on honeybee pollination or pollination by other bees (Roubik, 1995).

Pollinator changes include shifts in species composition of pollinators and reductions or even losses of pollinators. These changes can potentially have demographic and/or evolutionary consequences for plant populations. Demographics can be impacted if (1) the change increases pollen limitation of seed production, and (2) the lowered seed production alters population growth. If the rate of increase for the population drops below replacement, the result could even be local extinction, especially if seed production drops even farther in small populations (as in the Allee effect seen in *Banksia goodii* R. Br.; Lamont et al., 1993). Even if demographic changes are not seen, evolution could be altered if the new pollinator regime selects for different floral or reproductive traits.

pollen limitation

Most effort to date has focused on direct demographic changes, rather than evolutionary ones. The first requirement for a demographic change has considerable evidence. In the 1970s, it was proposed that seed production would rarely be pollen limited (Charnov, 1979; Willson, 1979). These influential papers were based on application of current sexual selection theory to plants and contributed, along with the conceptual papers by Raven mentioned above, to a renaissance in pollination ecology. Among other things, the notion of sexual selection spawned a large number of studies of pollen limitation (Burd, 1994). Pollen limitation can be demonstrated by an increase in reproduction when pollen loads on stigmas are supplemented by hand pollination, ideally of all flowers on a plant to rule out compensatory changes due to re-allocation of resources between flowers. A recent meta-analysis of studies in 306 species across 80 plant families found that fruit production is limited by receipt of low levels of pollen in the majority of cases (Knight et al., 2005), contrary to the original prediction from sexual selection theory. Furthermore, populations that have recently suffered habitat fragmentation have lower levels of pollination, as well as lower reproductive success (Aguilar et al., 2006). What we don't know in these cases is how critical seed production is to the growth of the population, the second requirement for a demographic change. A population that increases its seed production may nevertheless fail to thrive if a density-dependent process, such as competition of seeds for a limited number of suitable germination sites, negates the higher seed production. Several studies have combined the extent of pollen limitation with population projection matrices (Knight et al., 2005) in a first attempt to look at the demographic consequences; however, in all of these cases, absence of density-dependence was assumed.

experimental tests for demographic effects in *Ipomopsis*

My colleagues and I have begun to examine the possibility of demographic effects of low pollination in *Ipomopsis aggregata* and *I. tenuituba* (Price et al., 2008). In populations of *I. aggregata* near Gothic, Colorado, hummingbirds are responsible for about

94% of all flower visits made by pollinators (Price et al., 2005). These populations are often pollen limited, with supplemental hand pollination of all flowers on a plant approximately doubling total seed production (Campbell, 1991; Campbell & Halama, 1993). We simulated this increase experimentally by adding the estimated number of seeds produced by five naturally pollinated plants or five plants with supplemental hand pollination (750 or 1500 seeds) to areas of 8 m^2. Seeds were added again the next year to simulate persistent pollen limitation, and each treatment was replicated six times. Doubling the seed input to simulate full pollination roughly doubled the number of seedlings that emerged in each 8-m^2 plot (means = 132 vs. 252 seedlings) and the number of individuals that survived to flower (7 vs. 17 flowering plants; Price et al., 2008). Over this range of densities corresponding to natural versus full pollination, per-capita success was independent of density even though density dependence was evident over a wider 10-fold range of seed inputs.

To estimate the demographic effect of full pollination, we calculated the finite rate of increase (λ) for a population using the observed values of age-specific survival and age at flowering, while estimating age-specific fecundity as the observed proportion of surviving individuals that reproduced, multiplied by the average number of seeds made by either fully or naturally pollinated plants. A population would have $\lambda = 1.14$ (positive growth) under a scenario of full pollination versus $\lambda = 0.94$ (negative growth) under a scenario of continued natural pollination (Price et al., 2008). In this case, low seed production due to low pollen transfer does have a demographic impact, and natural populations may currently be in some peril from low pollination.

POTENTIAL DEMOGRAPHIC EFFECTS OF POLLINATOR SHIFTS

Applying this approach to *Ipomopsis tenuituba* allows us to consider the potential demographic consequences of a shift from hummingbird to hawkmoth pollination. The reciprocal transplant studies at Poverty Gulch discussed above have indicated that *I. tenuituba* may be in decline even in its native habitat. In 1994, we planted seeds from 10 full-sib families into a natural population of this species. The 197 seeds we planted eventually produced five plants that survived to reproduce (or that were still alive at the time our estimate was made in 2005), and produced an estimated 172 seeds themselves (details given in Campbell & Waser, 2007), giving a pooled net reproductive rate of $R_o = 0.87$ and a pooled finite rate of increase of $\lambda = 0.98$. These values for R_o and λ are based on small samples, but if taken at face value would lead *I. tenuituba* to be considered Vulnerable (VU) according to the IUCN Red List (IUCN, 2001). In addition, they were obtained in an experiment where seeds were planted just under the soil surface, which increases germination relative to seeds that are simply scattered as would occur naturally (M. Price, N. Waser, D. Campbell, A. Brody, unpublished data).

In part, the low success of *Ipomopsis tenuituba* can be attributed to low rates of hawkmoth visitation. Demographic studies indicate that the finite rate of increase for *Ipomopsis* is elastic to increases in fecundity (Campbell & Waser, 2007); a percentage increase or decrease in fecundity during the first big year of flowering (age five or six) can have a substantial effect on the finite rate of increase. Indirect evidence that the level of hawkmoth pollination influences fecundity comes from comparison of fruit sets in years with and without substantial numbers of hawkmoths. In the time span that we have studied this hybrid zone, hawkmoths were most abundant in 1993. Fruit set (% flowers that set fruit) that year in *I. tenuituba* populations was 90%, compared with 25% in 1992 and 26% in 2002, both years without hawkmoths (Campbell et al., 1997, unpublished data). Furthermore, in 1992, fruit set increased in clinal fashion from the high-elevation *I. tenuituba* populations to the lower-elevation *I. aggregata* populations where hummingbirds were more abundant, while no such cline was observed in 1993 (Campbell et al., 1997). These data suggest that high fruit set is obtained only in years when hawkmoths are present.

During the past 20 years, my colleagues and I have observed hawkmoths in substantial numbers in the surrounding area in only two years. It is possible that hawkmoth frequency was historically higher (Grant & Grant, 1965). The current rarity of hawkmoths in the higher mountains might conceivably be a recent anthropogenic change, perhaps from widespread use of insecticides in the desert areas, which harbor larger populations of these animals, and/or from other causes, although there are no data on population trends of moth species in the United States (National Research Council, 2007). Regardless of the reason for low abundance, it is useful to ask: would a higher abundance of hawkmoths maintain viable populations of *Ipomopsis tenuituba*? This question can be addressed by using a demographic transition matrix with elements corresponding to age-specific survival and fecundity, while also considering what happens when fecundity is multiplied by a factor that corresponds to frequency of years with hawkmoths. Assuming that fruit and seed set are linearly related to the frequency of years with hawkmoths, and fruit set is

k times as high when hawkmoths are present:

$$R_o = (1 - m)B + kmB$$

where m = frequency of years with moths and $B = R_o$ when only birds and no hawkmoths are present. In our case, $k = 0.90/0.25 = 3.6$. Noting that one out of 10 years in which plants bloomed in our experiment had hawkmoths ($m = 0.1$), and in that situation $R_o = 0.87$ seeds per seed planted, solving gives $B = 0.69$. Thus, we would expect $R_o = 0.69$ if hawkmoths were never present and $R_o = 2.49$ if hawkmoths were always present. It would take a minimum of two years out of 10 with hawkmoths to raise R_o above 1. This simplistic calculation ignores evolutionary change (see below) but suggests that these *I. tenuituba* populations are in some danger unless visitation by hawkmoths and/or hummingbirds increases. It is worth noting that this species combines a number of traits that could make it particularly vulnerable: self-incompatibility, a monocarpic life cycle with no seed bank, and some dependence on hawkmoths that are extremely variable from year to year. In North America, many species of butterflies and moths are regarded as threatened or endangered in various regions (National Research Council, 2007). While effects of such low pollinator numbers on plant populations are rarely studied, one exceptionally clear-cut case of endangerment due to low hawkmoth pollination is provided by the tree *Oxyanthus pyriformis* (Hochst.) Skeels in South Africa (Johnson et al., 2004).

We need many more such studies that combine pollination and demography in order to assess the severity of what some have referred to as a pollination crisis (Kearns et al., 1998). It is perhaps easier to list what we do know about the problem than what we don't know. However, from the plant side it is clear that little is known about demographic effects and about what types of plants will suffer the most. It is important to identify such situations in order to know where to focus conservation efforts, and this will likely be an area of much future work. Aguilar et al. (2006) found greater effect of habitat fragmentation on pollination for self-incompatible species, but the importance of most other traits has yet to be demonstrated.

An Integrative Evolutionary Perspective on the Conservation of Plant-Pollinator Interactions

Putting evolution into impact assessment

To date, most assessments of the impact of loss of pollinator have ignored the influence of evolutionary change on demographic processes. A few studies have considered the possibility that reduction in pollinators would alter selfing frequency and inbreeding depression (e.g., Lazaro & Traveset, 2006); however, most other genetic or evolutionary changes have been ignored. It is now abundantly clear that some natural populations experience selection strong enough (Endler, 1986) to produce evolutionary change on short time scales (Reznick & Ghalambor, 2001). Adaptation of populations to new selective regimes could, in principle, happen quickly enough to prevent extinction, and the potential for this process has been demonstrated theoretically (Gomulkiewicz & Holt, 1995). Still, empirical data are lacking, and the potential for such evolutionary changes has hardly been considered in any particular case, especially outside of the case of global climate change (Rice & Emery, 2003). We need to consider the possibility of such evolutionary change in the context of loss of pollinators.

An illustration based on *Ipomopsis tenuituba*

In this section, I will outline how we might consider the possibility of evolution in response to pollinator loss using the case of *Ipomopsis tenuituba*. Large variation in fitness among the 10 full-sib families we planted suggests a high opportunity for selection on traits that influence reproduction (Campbell & Waser, 2007). If hawkmoths disappeared entirely from these populations, could these plants evolve characters making them more attractive to hummingbirds quickly enough that the risk of extinction would be ameliorated? Selection on traits such as corolla width is strong, raising the possibility that an increase in corolla width might increase hummingbird visitation sufficiently to raise reproduction above the replacement rate. Of course, this scenario would require that hummingbird abundances not decline below their present level, and there are some suggestions of recent declines in the hummingbird pollinators as well (Nabhan, 2004). Even if it were possible for an evolutionary change in traits to increase hummingbird visitation, this would lead to greater similarity between the two species, and, thus diversity in a phenotypic sense would not necessarily be maintained. Most of the data necessary to model the question are available, but sample sizes are, in some cases, rather small, so that the quantitative answers will be extremely rough. This example should be viewed mainly as a guide to the approach.

The first step is to estimate how fast a character, such as corolla width, would change evolutionarily due to selection. According to a simple quantitative genetic framework, the change in corolla width between generations can be predicted by the strength of phenotypic selection on the trait (measured by the

selection differential S) multiplied by heritability of the trait. A more precise prediction can be made by taking into account genetic correlations between characters (Lande & Arnold, 1983), but for simplicity, those correlations will be ignored here. This procedure is equivalent to assuming that the selection by pollinators is only on corolla width and no other genetically correlated characters. Campbell (1996) measured the heritability of corolla width under natural conditions in *Ipomopsis aggregata* as 0.30, indicating that 30% of the variation could be explained by the average effects of alleles passed on by parents. Combining that figure with the phenotypic selection exerted by hummingbird visitation when presented with arrays of both *Ipomopsis* species and their hybrids (selection differential = 0.064; Campbell et al., 1997) predicts an evolutionary change of 0.02 mm per generation, about 1/50 of the difference in this trait between the two species. This calculation assumes heritability would be identical in *I. tenuituba* (a simplistic assumption; see Roff & Mosseau, 1999) and ignores any selection arising from greater efficiency of hummingbirds at dispersing pollen from wider flowers (Campbell et al., 1998). Furthermore, it assumes directional rather than stabilizing selection on the character, in contrast with Gomulkiewicz and Holt (1995), who envisioned a shift from one optimal value to another under the new selective regime. Incorporating stabilizing selection instead can also be done using simple quantitative genetics models, but that approach was rejected here because the observed phenotypic selection was primarily directional (Campbell et al., 1997).

The second step is to allow fecundity to increase in the next generation to the extent predicted by how the new phenotype (in this case a corolla that is 0.02 mm wider) would increase pollinator visitation. These data are available from studies of hummingbirds visiting flowers in experimental arrays of the two species and hybrids (Campbell et al., 1997). The net reproductive rate would increase between generations as follows:

$$R_o = B[(v + bzt)/v] = B\left[1 + \frac{bz}{v}t\right]$$

where B = net reproductive rate in the original generation following loss of hawkmoths, v = current hummingbird visit rate for *Ipomopsis tenuituba*, b = slope representing the increase in visit rate per change in corolla width, z = the change in corolla width per generation, and t = time in generations. The part in square brackets expresses the proportional increase in visit rate relative to the original rate by hummingbirds. Notice that what matters is the strength of selection and the response to selection (z) relative to the original visit rate. Campbell et al. (1997) found an increase of 0.18 visits per millimeter from a mean of 0.17 visits per flower per hour for *I. tenuituba* plants (Campbell et al., 1997: fig. 3). Substituting,

$$R_o = 0.69\left[1 + \frac{0.18(0.02)}{0.17}t\right]$$

$$= 0.69[1 + 0.021t].$$

This approach assumes that fecundity is linearly related to visit rate. This assumption is unlikely to hold exactly, as seed production is expected to level off at high pollen receipt (Ashman et al., 2004), but may be a reasonable first approximation in this system since even *I. aggregata*, with its much higher visit rate, suffers pollen limitation.

The final step is to iterate the population size using this dynamic model for R_o. According to this simple model, it would take 22 generations for corolla width (198 years for this plant with its generation time of nine years) to increase sufficiently in response to selection for the net reproductive rate to rise above 1 (Fig. 1A). Using this change in R_o, in the meantime, the population size would decline to 2% of its initial value (Fig. 1B, solid line). Even using a more

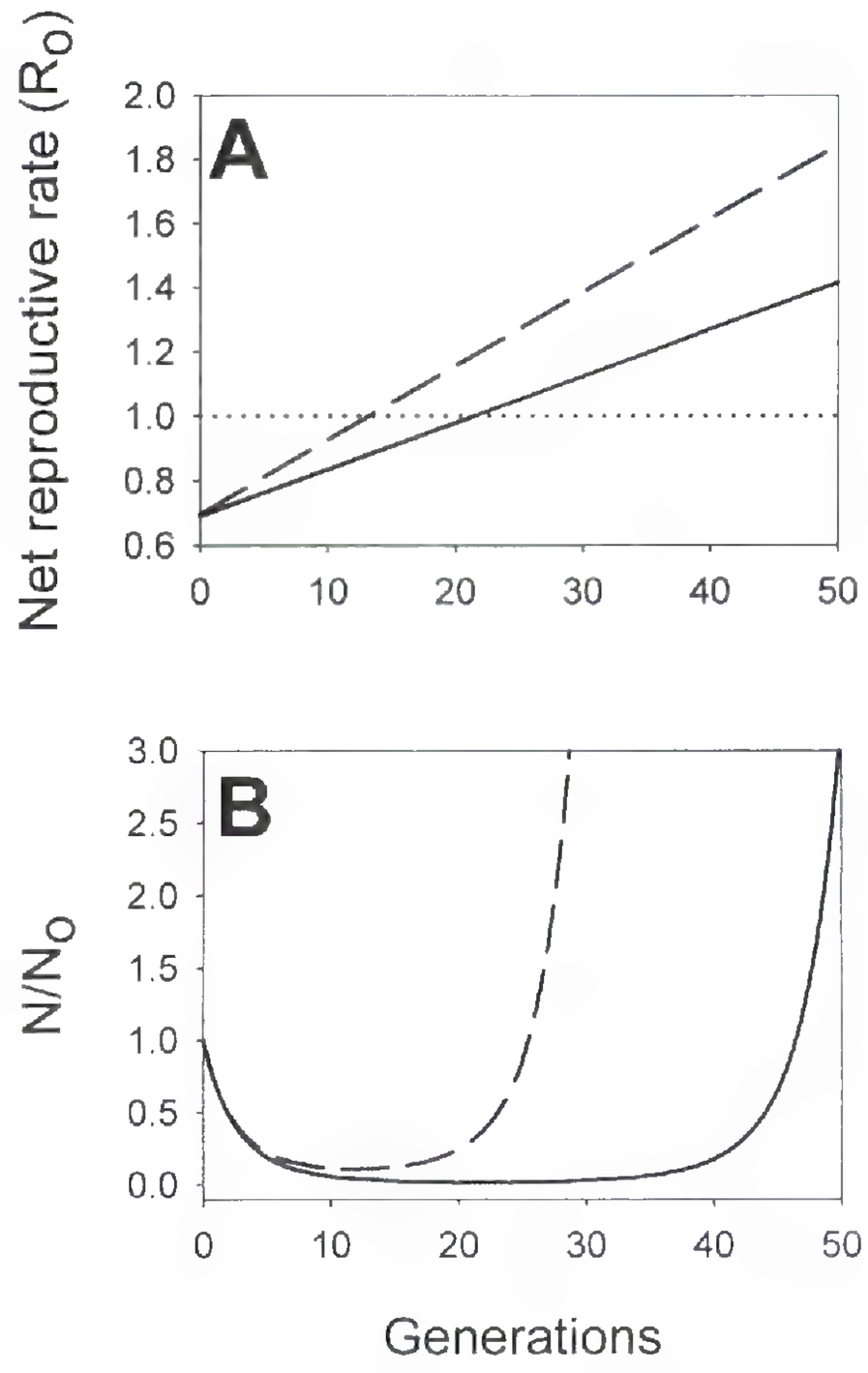

Figure 1. Estimated changes in the (A) net reproductive rate (R_o) and (B) population size (N) relative to its initial value (N_0), according to a model that allows for increased fecundity due to response to selection on a floral trait. Strength of selection (b) = 0.18 (solid lines) or b = 0.33 (dashed lines).

optimistic value for how visit rate would increase ($b = 0.33$ based on a measure of selection taking into account correlated effects of other characters; Campbell et al., 1997), the population size would decline to 11% of its initial value before starting to recover after 13 generations (Fig. 1). Given that the current size of the population is probably in the hundreds, not thousands, these calculations suggest the population would likely be vulnerable to extinction through demographic or environmental stochasticity before it could evolve a sufficient increase in pollination level, despite the strong selection on corolla width.

All of the above considerations ignore the potential effects of hybridization. Hybridization can impact a population in one of two ways. If hybrids are relatively unfit, hybridization can lower the net reproductive rate of the population. Second, even if hybrids are fit, they may show signs of introgression, changing the population through genetic assimilation, as has occurred for example in *Helianthus* L. (Carney et al., 2000) and *Spartina* Schreb. (Anttila et al., 2000). In the case of *Ipomopsis* hybridization, hybrids with *I. tenuituba* as the mother and *I. aggregata* as the father had zero fitness in the home site for *I. tenuituba*, so hybridization would likely contribute further to decline of the population rather than to genetic assimilation.

I stress that these predictions concerning the effects of loss of hawkmoths, even if taken at face value despite the uncertainty in estimates of parameters, would apply only to the potential of local extinction from the region near Poverty Gulch, not global extinction of the species. Pollinator populations vary greatly in space, and hawkmoth numbers, in particular, seem to be highly variable. For example, in the columbine *Aquilegia caerulea* James, hawkmoths accounted for 68% of flower visitation in an Arizona population, while being absent entirely from another population in Colorado near our study site (Brunet & Sweet, 2006). In other populations of *Ipomopsis tenuituba* studied at Grizzly Ridge, hawkmoth visitation was 50% higher than at Poverty Gulch, which might be sufficient to maintain viable populations (Aldridge & Campbell, 2007).

Conclusions and Future Directions

Since the publication of important conceptual papers by Raven in the 1970s, pollination ecologists have made much progress in understanding the evolution of floral diversity. In a number of cases, the influence of pollinators on microevolution of floral traits has been documented, and even quantified, through estimates of selection and heritabilities (Galen, 1996; Ashman & Majetic, 2006). Much recent attention has been directed at the origin and maintenance of floral differences between species, and progress in this area will undoubtedly continue as new genetic tools and methods of analysis allow a finer look at the genetic basis of the traits (Bradshaw et al., 1998) and their fitness consequences (Morgan & Conner, 2001). We have come a long way in understanding how changes in pollination regimes can lead to diversification and origin of species. Much less, however, is known about how recent changes in pollination regimes impact the demographics of plant populations and might thereby lead to loss of plant species. Given the mounting evidence for decline of some pollinators, there is urgent need to design experiments that evaluate whether lower pollination will result in smaller populations of wild plants, particularly for those species that are already threatened. Such studies should consider not only direct demographic effects of lower pollination and seed production, but also the possibility of indirect demographic effects that occur through rapid evolutionary changes in trait values or in levels of inbreeding. It is time to heed Raven's (2002) call for action and bring the growing knowledge of ecology and evolution of pollination to bear on evaluating the impact of anthropogenic changes in populations of animal pollinators.

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A RENAISSANCE OF CYTOGENETICS: STUDIES IN POLYPLOIDY AND CHROMOSOMAL EVOLUTION[1]

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ABSTRACT

Systematics and evolutionary biology are being bolstered by a renaissance in cytogenetics and comparative genomics as illustrated by reviewing Peter Raven's integration of cytogenetics and phylogenetics and by presenting updates to his work in three key research areas. The first area is the evolution of chromosomes during the origin of the angiosperms. Raven's analysis of chromosome numbers in the Annonales and other basal angiosperms inspired modern genomic comparisons that have revealed paleopolyploid events, which appear to have occurred early and often during flowering plant diversification. Second, Raven's characterization of chromosome evolution in various genera of Onagraceae is updated in light of a contemporary Onagraceae phylogeny. The possible construction of ancestral karyotypes in the Onagraceae is feasible using techniques that have been successful in analyses of genomic blocks in the Poaceae and Brassicaceae. Third, Raven's work on catastrophic speciation identified the importance of chromosomal rearrangements in the evolution of *Clarkia* Pursh and the ability of new species to inhabit different environments. Current work in *Brassica* L. has shown that phenotypic changes contributing to speciation events can arise from relatively few chromosomal rearrangements. A fusion of systematics and cytogenetics is opening new areas of research, with phylogenomics allowing ancestral genome reconstruction, the incorporation of genome-level characters into phylogenetic analyses, and new theories about evolution on a genomic scale.

Key words: *Brassica*, Brassicaceae, chromosome evolution, *Clarkia*, molecular cytogenetics, Onagraceae, phylogenetics, phylogenomics, Poaceae, polyploidy.

In 1986, Peter Raven commented, "a genuine conceptual difficulty with the field of biosystematics is that it was always conceived as a way of improving plant classification, rather than a way of learning more about plant evolution" (Raven, 1986: 26). Indeed, the history of biosystematics itself is problematic. The origin of phylogenetics in the mid-20th century caused drastic changes in how systematics is viewed philosophically. Before the advent of phylogenetics in the mid-20th century, biosystematists had concentrated on closely related species and within-species variation, with methodologically and theoretically flexible views of breeding and chromosomes, viewing evolutionary relationships as webs. The arrival of tree thinking switched the focus of systematics primarily toward resolving phylogenetic relationships of more distantly related species and higher taxa, with a more monistic interpretation of traits and homology (Sytsma & Pires, 2001). In 2006, Peter Raven remarked in personal communication, "the two biggest advances in my lifetime were (1) the conceptual distinction between synapomorphy and pleisiomorphy and (2) the development of technology to allow us to look at chromosomes, then flavonoids, then isozymes, and finally DNA." The most important advancements over the past several decades were improved methods of building trees and visualizing phylogenetic relationships. While the importance of phylogenetics need not be questioned, we can ask whether its growth comes at the expense of other disciplines, such as evolutionary development and cytogenetics. The past few decades have shown how a field such as evolutionary development (evo-devo) can emerge, suggesting the potential for resurgence of other fields (Love, 2003; Breidbach & Ghiselin, 2007; Muller, 2007; Pigliucci, 2007).

Here, we argue that a similar renaissance in cytogenetics and comparative genomics is occurring

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with the advent of modern cytogenetic techniques and methods for analyzing genomes. We illustrate this by reviewing Peter Raven's integration of cytogenetics and phylogenetics and by presenting updates to his pivotal work in three key research areas. First, how can the chromosome numbers of basal angiosperms contribute any information to chromosome evolution across angiosperms? Raven's investigation of chromosome numbers in the Annonales supports subsequent conclusions about the basal chromosome number of angiosperms, and these analyses inspired genomic comparisons that revealed paleopolyploid events. Second, how have chromosomes evolved in Onagraceae? Raven's characterization of the types of chromosomes found in various genera of Onagraceae reveals interesting patterns of chromosomal evolution when mapped onto the contemporary Onagraceae phylogeny. Recent advances in similar work allow the construction of ancestral karyotypes in grasses and analyses of genomic blocks in Brassicaceae. Finally, how have chromosomal rearrangements led to rapid speciation in the genus *Clarkia* Pursh? Work in catastrophic speciation, which occurs when a minority cytotype becomes adaptively advantageous following dramatic habitat change, allowed Raven to identify the importance of chromosomal rearrangements in *Clarkia* speciation, and new examples from the same genus highlight the abilities of these species to inhabit different environments. Current work in *Brassica* L. has consequently shown that relatively few chromosomal rearrangements can lead to phenotypic changes that may contribute to speciation events.

Basal Chromosome Number in the Angiosperms

From a phylogenetic viewpoint, the term basal is used to denote organisms that are placed at or near the base of a phylogenetic tree, and is always used in reference to a certain group of organisms. When discussing deep phylogenies of angiosperms, authors often refer to groups of organisms whose branches originate near the base of the tree, such as *Amborella* Baill. and the Magnoliales, as basal angiosperms. In fact, these plants are merely descendants of the true ancestral angiosperms, which are presumably now extinct (Crisp & Cook, 2005). Indeed, *Amborella* and all extant flowering plant lineages are equally distant from a common ancestor. For the purposes of this paper and in concordance with the literature being reviewed, the lineages of plants that diverged from a common angiosperm ancestor prior to the monocot-eudicot split will be referred to as basal angiosperms, with the understanding that collectively they are merely extant approximations of the true ancestors of flowering plants.

One of Raven's earliest contributions to cytology involved the basal chromosome number in the angiosperms (Raven & Kyhos, 1965), and these conclusions have been updated by more recent chromosome counts (Soltis et al., 2003). The advent of genome sequencing has consequently allowed comparisons between species for more thorough analyses of chromosomal evolution. It was Raven's thought that chromosome counts from the Annonales, or the woody Ranales, might shed light on angiosperm base chromosome number. His 1965 paper published the chromosome counts for *Degeneria* I. W. Bailey & A. C. Sm. ($n = 12$) and *Drimys* J. R. Forst. & G. Forst. (43 bivalents). These counts led Raven and Kyhos (1965: 247) to state, "with our present information, it appears very likely that $n = 7$, as suggested by Darlington (1956) is the original base chromosome number of angiosperms." This conclusion seemed fairly reasonable; a relatively low base chromosome number is selectively advantageous because it reduces recombination (Raven & Kyhos, 1965: 247). However, Raven cautioned that aneuploidy and polyploidy confound the issue, and phylogenetic considerations should be applied to interpret the base chromosome number of angiosperms. Moreover, "there are still six families of annonalean affinities for which not a single chromosome count is available" (Raven & Kyhos, 1965: 247). Raven's interest in comprehensive chromosome counting did not stop with this paper, however; he was instrumental to the founding of the Index to Plant Chromosome Numbers (IPCN Series), which originated at the University of California, Berkeley, with Marion Cave's efforts in 1956, but moved to the Missouri Botanical Garden in 1978.

Subsequent research in major patterns of cytology and phylogeny in the angiosperms has confirmed the relevance of Raven's conclusions regarding basal chromosome numbers in angiosperms. Major developments facilitating current advancement include a more accurate phylogeny of angiosperms and more chromosome counts from taxa of early diverging lineages. Subsequent researchers have continued to fill in gaps in chromosome numbers to provide a more complete picture of chromosome evolution in angiosperms (Ehrendorfer et al., 1968; Walker, 1972; Ehrendorfer & Lambrou, 2000). Additionally, we are gaining a better understanding of relationships among the basal angiosperms; current phylogenies place *Amborella* and *Nuphar* Sm. as sister groups to the rest of the flowering plants. Paradoxically, many basal angiosperms are now known to have high haploid chromosome numbers (i.e., *Amborella* with $n = 13$ [Oginuma et al., 2000], *Illicium* L. with $n = 11$ [Fedorov, 1969], Magnoliaceae with $n = 19$ [Fedorov, 1969]). Despite these observations, current thought

indicates the original base chromosome number is low (x = 6–9) (Raven, 1975; Soltis et al., 2003, 2005).

Current methods of characterizing genome evolution go beyond mapping chromosomes onto phylogenies by exploring the prevalence of polyploidy and other genome duplications in angiosperms. A variety of research methods, including isozymes and phylogenomics, suggest that genome duplications have arisen in many angiosperm families, with repeated cycles of polyploidization and diploidization. Classic estimates of genome evolution in polyploidy indicate 30%–50% of extant angiosperms are polyploids, and recent studies suggest that most extant angiosperms share at least one polyploid ancestor (Cui et al., 2006). Isozymes first detected duplicated loci in Magnoliaceae and other basal lineages, providing the first suggestion of paleopolyploid events; isozyme evidence for duplications in eudicots confirmed earlier hypotheses of polyploidy in angiosperms (Soltis, 2005).

A variety of inventive methods of genome analysis have been applied to detect the history of whole-genome duplications in angiosperms. Bowers (2003) fused phylogenetics and genomics to evaluate ancient genome duplications within *Arabidopsis* (DC.) Heynh. and found three nested polyploid events. The ages of duplicated genes suggest an alpha duplication event after the Brassicaceae-Malvaceae split, an older beta duplication event after the monocot-eudicot split, and perhaps a more ancient gamma duplication event that is difficult to place. Comparisons of sequenced genomes reveal duplicated genes, which provide evidence of ancient polyploidy in *Arabidopsis*, *Oryza* L., and *Populus* L. (Blanc et al., 2003; Paterson et al., 2004; Yu et al., 2005). In a parallel effort, Floral Genome Project investigators examined basal angiosperm lineages for older polyploidy events that predated the monocot-eudicot divergence. Ks analyses revealed independent ancient polyploidy events in several early diverging angiosperm lineages. Much slower rates of sequence evolution occur in basal angiosperms, especially *Liriodendron* L. (Magnoliaceae), allowing analyses to determine that at least one ancient polyploidy event occurred in the common ancestor of all angiosperms or in the common ancestor of all angiosperms other than *Amborella* (Cui et al., 2006).

Raven's early work counting chromosomes in the Annonales allowed him to make hypotheses about the base chromosome number for all angiosperms. This type of work laid the foundation for more thorough analyses of chromosome number with the discovery of other basal angiosperm lineages, which, in turn, have advanced more in-depth investigations of chromosomal evolution and ancestral karyotype inferences via phylogenomic comparisons.

Chromosome Evolution in Onagraceae

Although Peter Raven's ideas about basal angiosperm chromosome number were insightful, he is perhaps more famous for his work on chromosome evolution within the Onagraceae, the evening primrose family. His initial characterization of Onagraceae chromosomes eventually led to hypotheses about the phylogenetic patterns in the family, and current work on ancestral karyotype reconstruction in the Poaceae and Brassicaceae reaffirms the importance of addressing chromosomal organization in a phylogenetic framework.

An early collaborative work on mitosis in the Onagraceae covered all tribes, 15 genera, and 46 species. This comparative work described three modally distinct groups of chromosomes that vary in size and heterochromatic content. Members of the first group of chromosomes are presumably ancestral and medium sized, contract evenly along length during meiosis, and are not visible in interphase; they are found in *Fuchsia* L., *Circaea* L., and *Lopezia* Cav. The second group is intermediate in age; these chromosomes are morphologically dot-like and heteropycnotic throughout meiosis, and are not visible in interphase; they are found in *Ludwigia* L., *Epilobium* L., and *Boisduvalia* Spach. Chromosomes in the third group are derived, medium sized, and heteropycnotic near the centromere, and have long, lightly staining euchromatic tails; they are found in *Hauya* DC. and *Onagreae*. These groupings were used to support *Fuchsia* and *Ludwigia* as less-derived genera of the family (Kurabayashi et al., 1962).

When combined with Raven's classification of Onagraceae chromosomes, the current Onagraceae phylogeny (Levin et al., 2003) suggests subtle patterns about chromosome evolution. The previously mentioned chromosome types (Kurabayashi et al., 1962) do not map simply onto the Onagraceae phylogeny; small, dot-like chromosomes have evolved independently multiple times. Later karyomorphological studies in the Onagraceae described four types of chromosomes (Tanaka et al., 1988). There were two major differences between the old and new chromosome groupings. In the new chromosome classification scheme, the genera of section *Onagreae* were split into two groups, and both the first and second groups in the original classification were reorganized. This new chromosome classification scheme suggests variation in the origin of chromosome morphology and indicates that cytology of genera reflects phylogenetic relationships. For example, the independent evolution of small chromosomes in Onagraceae can be explained by more thorough analyses of chromosome structure. Although *Epilobium* and *Ludwigia* both have small

chromosomes, *Epilobium* chromosomes seem to be a specialization of more diffuse (type 1) chromosomes (P. Raven, pers. comm.).

Recent comparative genomic analyses of the Poaceae and Brassicaceae are illustrative of potential future practices in family-wide plant systematic studies. Comparisons among grass genomes indicate that there is colinearity among the grass genomes with rice as the central reference genome (Devos et al., 2002). The benefits of this comparative approach (e.g., using synteny to find candidate genes in non-model taxa) make it a desirable analytical tool to apply to other taxonomic groups with copious genetic information available. For example, is it possible to compare colinearity among crucifer genomes with *Arabidopsis thaliana* (L.) Heynh. as a reference? Although *A. thaliana* will continue to be the functional reference point, its small, derived genome ($n = 5$) complicates larger-scale structural genomic comparisons to other members of the Brassicaceae. Given that complete genome sequences for at least four crucifers (*A. lyrata* (L.) O'Kane & Al-Shehbaz [$n = 8$], *Capsella* Medik. [$n = 8$], *Brassica rapa* L. [$n = 10$], and *Thellungiella* O. E. Schulz [$n = 7$]) will be available within a few years, it is unlikely that *A. thaliana* alone will continue to be used for structural comparisons. It may be possible for genetic maps and cytogenetic data from several Brassicaceae genomes to be combined to create a generalized reference set of ancestral genomic blocks. The *A. thaliana* genome can be split into at least 21 conserved blocks, and comparative analyses reveal *A. thaliana* and *B. napus* L. share large, colinear blocks that have undergone numerous rearrangements (Parkin et al., 2005). These types of structural comparisons allow extrapolations about the evolution of more derived states. In fact, the karyotype of *A. thaliana* is derived from a tentative $n = 8$ ancestral karyotype; genetic maps of closely related species, *A. lyrata* and *C. rubella* Reut., are collinear ($n = 8$) and show how *A. thaliana* ($n = 5$) is rearranged and derived (Lysak et al., 2006). Investigating the *A. thaliana* and *Brassica* genomic blocks with other crucifer genomes will allow for the reconstruction of an ancestral karyotype, which will better facilitate comparisons across the Brassicaceae (Schranz et al., 2006).

Peter Raven's analysis of chromosomal forms in Onagraceae provided invaluable information to researchers who could later analyze chromosomal evolution in the family. Current research in Poaceae and Brassicaceae has built on similar types of analyses by describing how genomic blocks can show conserved and derived patterns of chromosomal evolution across closely related species. These studies indicate a major role for cytogenetic data in phylogenomic comparisons. At the family level, analyses of chromosomal blocks and ancestral karyotype reconstruction will most likely be developed first in Poaceae, Brassicaceae, Leguminosae, Solanaceae, and Asteraceae. However, as genomic resources and comparative analyses improve, Onagraceae, and any other family of choice, will not be far behind. One can imagine the possibility of generating ancestral genome browsers for deeper-nested nodes (e.g., Brassicales, rosids, eudicots, angiosperms) that would allow for a myriad of phylogenetic inferences and comparisons across clades of flowering plants.

Rapid Speciation in the Genus *Clarkia* and Chromosomal Rearrangements

Peter Raven's research interests not only spanned comparisons of orders and families of flowering plants but also involved integrating phylogenetics and cytogenetics within genera and species. Specifically, Raven's theories about catastrophic selection and edaphic endemism as a factor in speciation later informed phylogenies about *Clarkia* and modes of adaptation to habitats. Modern research incorporating cytogenetics and phenotype change in *Brassica* confirms the ability of species to adapt quickly to new environments and to become reproductively isolated.

Early work by Lewis (1962) theorized that the removal of most of a population would allow a deviant, or better adapted, genome to establish a new marginal population. This concept of catastrophic speciation was later expounded upon by Raven (1964: 338) when he suggested that "catastrophic selection and consequent genotype reorganization may be a potent force in the creation of new edaphically restricted endemics." He noted a pattern possibly supporting this hypothesis in *Clarkia rubicunda* (Lindl.) F. H. Lewis & M. E. Lewis and *C. franciscana* F. H. Lewis & P. H. Raven, but found further support in distinctive endemics like *Microcycas* (Miq.) A. DC. (Mason, 1946) and narrow endemics like the *Streptanthus glandulosus* Hook. complex (Kruckeberg, 1957).

Recent phylogenetic and cytogenetic analyses of *Clarkia* reveal fascinating patterns of speciation and chromosomal evolution. Although very closely related with little sequence divergence, *Clarkia biloba* A. Nelson & J. F. Macbr. ($n = 8$) and *C. lingulata* F. H. Lewis & M. E. Lewis ($n = 9$) are effectively reproductively isolated due to hybrid sterility; their differences in base chromosome numbers result in poor chromosome pairing (Sytsma & Smith, 1992). In contrast, *Clarkia* sect. *Rhodanthos* subsect. *Rhodanthos* presents a more complicated example. Several researchers have utilized different types of data to determine the mode of speciation in this group.

Subsequent models of speciation project modes of speciation at different points in time (Lewis & Raven, 1958; Gottlieb, 1973; Sytsma et al., 1990; Gottlieb & Edwards, 1992). *Clarkia*, therefore, provides an interesting and complex system in which to examine chromosomal speciation, as it involves several modes of chromosomal evolution: shifts in base chromosome number, genome downsizing, and polyploid speciation.

While work on *Clarkia* has revealed the formation and establishment of taxa through catastrophic speciation, newer genomic methods are beginning to sort out mechanisms through which this process can occur. Work in diploid *Helianthus* L. hybrids (Rieseberg et al., 2003) and allopolyploid *Brassica* (Pires et al., 2004; Gaeta et al., 2007) suggests molecular mechanisms that can generate rapid variation, which may support the ecological theory of catastrophic speciation. A modern application of Peter Raven's work on speciation in *Clarkia* is found in analyses of resynthesized *Brassica* allopolyploids, which show rapid chromosomal and phenotypic change. In brief, chromosomal rearrangements caused by homoeologous recombination can alter the dosage of homoeologous alleles. This variable dosage of alleles can result in novel variation for flowering time, and genotypes with rapidly diverging flowering times can become isolated in the absence of cross pollination of early and late flowering groups. These studies of resynthesized *B. napus* polyploids elucidate potential mechanisms of speciation because rapid changes in genome structure alter gene expression and result in phenotypic variation of important life history traits like flowering time (Pires et al., 2004; Gaeta et al., 2007).

Initial work by Peter Raven on catastrophic selection in *Clarkia* suggested that dramatic changes in phenotype could arise from chromosomal changes and become fixed in a population; later work in *Clarkia* reaffirmed this pattern throughout the genus. Current research in taxa like *Helianthus* and *Brassica* also reveals that chromosomal rearrangements can cause phenotypic change that may eventually lead to speciation.

Toward a Cytogenetic Renaissance in Systematics

Peter Raven left his mark on several areas of research that have been continually reexamined and expanded. His ideas regarding the basal chromosome number in angiosperms were eventually confirmed by updated phylogenies and corresponding chromosome counts of newly identified basal angiosperm lineages. In turn, these studies have led to genome-wide investigations that have detected signatures of ancient polyploidy events occurring throughout the diversification of the angiosperms. Raven's characterization of chromosome types in Onagraceae has revealed complex evolutionary patterns in the family; these types of analyses have been extended in grasses and Brassicaceae to show how chromosomal blocks can be compared between organisms. Raven's work on catastrophic selection in *Clarkia* provided the theoretical framework for further research on the molecular mechanisms of chromosomal rearrangements and rapid speciation.

The revolution in evolutionary development was made possible by the formation of new, accessible methods of experimentation and analysis. Similarly, the cytogenetic renaissance will depend upon new methods that facilitate combining cytogenetic and phylogenetic analyses. The key to ushering forward the cytogenetic renaissance will arise from conceptually linking cytogenetics and phylogenetics by utilizing new cytogenetic tools, improving sequencing technologies, and cultivating phylogenomics (including comparative chromosomics) as a research priority (Dobigny et al., 2004; Graphodatsky, 2007).

First, new cytogenetic tools and applications will make cytogenetic research more accessible. Repetitive DNA sequences, like retroelements, are very useful for easily labeling chromosomes from parental species in a hybrid. Fluorescent in situ hybridization (FISH) probes for retroelements, as well as many other types of probes, can be quickly obtained from a variety of library-building techniques. One relatively inexpensive and efficient protocol for building small insert libraries (about 1000 bp) produced probes that effectively labeled retroelements from the genera *Zea* L. and *Tripsacum* L. (Lamb & Birchler, 2006). New techniques in FISH are allowing easy visualization of gene clusters or even single genes (Kato et al., 2006); regions as small as 3 kb can be effectively probed with these methods. These new developments are making cytogenetics accessible and useful for anyone interested in chromosomal evolution.

Second, new sequencing technology will alter the availability of data for phylogenomic analyses. Genomic sequences for select species are allowing more rapid development of nuclear gene loci that could potentially be used for phylogenetic analyses (Small et al., 2004; Gilchrist et al., 2006; Bouck & Vision, 2007). Complete genome sequences can be found in nearly every plant group (gymnosperms, moss, fern, fungi, algae), allowing all plant biologists to take advantage of this information. The use of genomic data for systematics will initially derive from mining complete genome sequences from closely related crop and model species, but the advent of low-cost genome sequencing technology and phyloge-

netically informed genomic comparisons (e.g., Rokas et al., 2003; Sjolander, 2004; Delsuc et al., 2005; Koonin, 2005; Philippe et al., 2005; Jeffroy et al., 2006; Jansen et al., 2007; Philippe & Blanchette, 2007) will provide resources for many species in question.

Finally, the cultivation of phylogenomics as a research area will actively integrate cytogenetics and genomics with phylogenetics. Phylogenomic analyses will ultimately establish not only evolutionary relationships among organisms but also relationships among all genes within an organism. A phylome, the complete collection of all gene phylogenies in a genome, has been developed for humans (Huerta-Cepas et al., 2007) and in the future will be developed for plants. Currently available resources such as bacterial artificial chromosome (BAC) libraries and expressed sequenced tag (EST) libraries can both be used by systematists to assess gene copy number and integrate cytogenetic data and nuclear genes with phylogenies. For example, Pires et al. (2006) utilized genome size and chromosome number to explore genome evolution in Asparagales and proposed that far more information could be unearthed using BACs or ESTs from crop plants like asparagus and onion to investigate non-model taxa like hyacinths, agave, amaryllids, irises, and orchids. The proliferation of genomic resources coupled to a fusion of systematic and cytogenetic methodologies promises to open up whole new areas of research. Phylogenomics will allow for ancestral genome reconstruction, the incorporation of genome-level characters into phylogenetic analyses, and new theories about evolution on a genomic scale.

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TAXONOMIC REVISION OF *ROLDANA* (ASTERACEAE: SENECIONEAE), A GENUS OF THE SOUTHWESTERN U.S.A., MEXICO, AND CENTRAL AMERICA[1]

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Abstract

A revision of the genus *Roldana* La Llave (Asteraceae: Senecioneae) is given, including a key to species and complete nomenclature with synonymies, description, distribution, and discussion sections for 48 species; eight varieties are recognized and 11 new combinations are made, including one species transfer to the genus *Psacaliopsis* H. Rob. & Brettell. A phytogeography section for the genus, which is distributed throughout the highlands of Mexico and Central America, with one species entering the southwestern-most U.S.A., is also included. New combinations are established for *R. acutangula* (Bertol.) Funston, *R. aliena* (B. L. Rob. & Seaton) Funston, *R. hartwegii* var. *carlomasonii* (B. L. Turner & T. M. Barkley) Funston, *R. hartwegii* var. *durangensis* (H. Rob. & Brettell) Funston, *R. hartwegii* var. *subcymosa* (H. Rob.) Funston, *R. kerberi* var. *calzadana* (B. L. Turner) Funston, *R. kerberi* var. *manantlanensis* (R. R. Kowal) Funston, *R. petasitis* var. *cristobalensis* (Greenm.) Funston, *R. petasitis* var. *oaxacana* (Hemsl.) Funston, *R. petasitis* var. *sartorii* (Sch. Bip. ex Hemsl.) Funston, as well as for the excluded taxon *P. pinetorum* (Hemsl.) Funston & Villaseñor. Neotypes are designated for *Cineraria platanifolia* Schrank, *R. ehrenbergiana* (Klatt) H. Rob. & Brettell, *R. lobata* La Llave, *R. petasitis* (Sims) H. Rob. & Brettell, *Senecio acerifolius* K. Koch, *S. canicidus* Sessé & Moc., and *S. prainianus* A. Berger. Lectotypes are designated for *Cacalia nutans* Sessé & Moc., *C. peltata* Sessé & Moc., *P. pinetorum*, *R. albonervia* (Greenm.) H. Rob. & Brettell, *R. aschenborniana* (S. Schauer) H. Rob. & Brettell, *R. gilgii* (Greenm.) H. Rob. & Brettell, *R. heracleifolia* (Hemsl.) H. Rob. & Brettell, *R. heterogama* (Benth.) H. Rob. & Brettell, *R. kerberi* (Greenm.) H. Rob. & Brettell, *R. langlassei* (Greenm.) H. Rob. & Brettell, *R. lanicaulis* (Greenm.) H. Rob. & Brettell, *R. petasitis* var. *cristobalensis*, *R. petasitis* var. *sartorii*, *S. brachyanthus* Greenm., *S. chapalensis* var. *areolatus* Greenm., *S. chrismarii* Greenm., *S. ghiesbreghtii* Regel var. *pauciflorus* J. M. Coult., *S. grandifolius* Loes. var. *glabrior* Hemsl., *S. hederoides* Greenm., *S. hypomalacus* Greenm., *S. lobatus* Sessé & Moc., *S. macrobotrys* Hemsl., *S. rotundifolius* Sessé & Moc., and *S. schumannianus* S. Schauer.

Key words: Asteraceae, Central America, Compositae, Mesoamerica, Mexico, *Roldana*, Senecioneae, taxonomy.

The genus *Roldana* La Llave (Asteraceae: Senecioneae) is distributed throughout the highlands of Mexico and Central America. It is placed in the subtribe Tussilagininae because it has stigmatic surfaces united across the inner face of the style branches, upper part of stamen filaments (anther collar) cylindrical, chromosome numbers $n = 30$, and principal phyllaries often with the midrib notably thickened at the base. The other tussilaginoid genera with which it is associated, based on geographic distribution, are *Digitacalia* Pippen, *Psacalium* Cass., *Robinsonecio* T. M. Barkley & Janovec, *Pippenalia* McVaugh, *Psacaliopsis* H. Rob. & Brettell, *Villasenoria* B. L. Clark, *Pittocaulon* H. Rob. & Brettell, *Telanthophora* H. Rob. & Brettell, *Nelsonianthus* H. Rob. & Brettell, and *Barkleyanthus* H. Rob. & Brettell. It is separated from these genera because it arises from a hairy, tuberous caudex with numerous fleshy roots; leaves typically palmate or, if pinnate, then typically longer than 10 cm and/or variously hairy. In this synopsis, 48 species and eight varieties are recognized and 11 new combinations are made, including one species transfer to the genus *Psacaliopsis*.

The genus has two centers of diversity; one is along the Trans-Mexican Volcanic Belt and Sierra Madre del Sur of south-central Mexico and the other is along the Sierra Madre de Chiapas and Cordillera Centroamericana of Chiapas and Central America. The species of *Roldana* vary in growth form from small perennial herbs that seldom exceed 1 m in height to freely branching trees that may reach 12 m in height. The architecture of the plants is insufficiently well understood to apply the rather precise descriptive

[1] If I were to properly recognize all those who helped with this revision, it would surely take as many pages; in memoriam, I thank my mother Sandra Scharbach Funston and my major professor Theodore Mitchell Barkley for their guidance of this work. Financial support was provided by the Division of Biology, Kansas State University, and the Missouri Botanical Garden.

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terminology of contemporary ecology, e.g., Hallé et al. (1978), although the utility of such a classification scheme is acknowledged. The genus is predominantly made up of perennial suffruticose herbs that may grow to shrubs. There are also four species of acaulescent perennial herbs and nine species that may grow to trees; stems are solitary or in clumps. Plants are typically distributed in the understory of pine-oak forests at elevations between 1000 and 4000 m. Herbaceous plants are also found along the forest edge in patches of disturbed grasslands. All species may be found in disturbed areas and roadsides. The main characters used to separate the species are habit, type and density of pubescence, number of phyllaries, and, to some extent, the shape of the leaf blade and its form of attachment.

In 1901, J. M. Greenman wrote a revision of *Senecio* L. s.l. from North and Central America. In his revision, he separated the genus into 22 sections. Since that time, there has been considerable debate whether the genus should remain together or be split into segregate genera. Currently, Greenman's concept of *Senecio* is split into two subtribes, with 11 genera from Mexico and Central America placed in the subtribe Tussilagininae. While Robinson and Brettell (1973a, b, c, 1974) made a comprehensive review of these genera that included keys, none was given a complete revision.

Materials and Methods

Information and materials were derived from field experiences in Costa Rica in January 1994, and Mexico in March 1996, from consultations in CR, IEB, MEXU, MO, and from herbarium specimens that were lent to KSC from A, ASU, CAS, DS, F, GH, KANU, MEXU, MICH, MO, NMC, NY, OS, SD, TEX, UC, UMO, and XAL—herbarium abbreviations follow Holmgren et al. (1990). Herbaria referred to, but from which no loans were requested, are B, C, G, K, P, and US. Gibson (1969: 97) notes in his dissertation, "Correspondence with Dr. G. Wagenitz indicates that all of the types of the genus *Senecio* at the Berlin Herbarium were destroyed in 1943." Species are presented in alphabetical order. Complete lists of exsiccatae and distribution maps are available from the author.

Microcharacters as reviewed by King and Robinson (1970), Jeffrey (1987, 1992), and Nordenstam (1978) have proven useful in the Senecioneae. Microcharacter studies of *Senecio* include Drury and Watson (1965), Jeffrey et al. (1977), Jeffrey (1979), Vincent et al. (1992), and Wetter (1983). For my dissertation (Funston, 1999), microcharacters in *Roldana* were investigated for consistency within the genus. Not all species could be coded; however, those that were showed a significant degree of similarity. While microcharacters did confirm the placement of species in the subtribe Tussilagininae, they were not sufficient to include or exclude a species at the generic level. A character state matrix of *Roldana* for Jeffrey's (1987, 1992) analytical descriptors is available from the author.

A numerical analysis performed on the species *Roldana hartwegii* (Benth.) H. Rob. & Brettell is also available from the author. A data matrix of 64 collections and five character states was analyzed using NTSYS-PC v. 1.8 (Rohlf, 1993). The data matrix was standardized using a simple matching coefficient. The standardized matrix was then clustered using the unweighted pair-group method, arithmetic average. The product moment correlation was used to obtain a cophenetic correlation of $r = 0.84$.

Taxonomic History

The genus *Roldana* stems from *Senecio* sect. *Palmatinervii* Hoffman (1894), a portion of the section *Fruticosi* Greenm. (1901), and two genera, *Pericalia* Cass. (1924) and *Digitacalia* (1968), from the old "cacalioid" complex of Mexico and Central America. The derivation of the genus is representative of the tortured struggle to segregate natural groups from the ubiquitous and heterogeneous *Senecio* s.l.

In Engler and Prantl, Hoffman (1894) defined palmately veined senecios of Central America as forming the section *Palmatinervii*. He did not list any species. Greenman composed a monograph of the North and Central American species of *Senecio*, which was published in his doctoral dissertation (1901) and through a series of papers in the *Annals of the Missouri Botanical Garden* (1914, 1915). In his dissertation, he divided the genus into 22 sections that included the sections *Palmatinervii* and *Fruticosi*. A revision of the sections *Palmatinervii* and *Fruticosi* was never completed. These were included in the subgenus *Eusenecio*, that is, with style branches truncate, rounded-obtuse, or occasionally terminated by a penicillate tuft of hairs; stems erect or ascending; stems without a foreshortened axis; oil tubes not richly developed in the cortex. The *Fruticosi* consisted of 13 species of pinnately veined shrubs or small trees. The *Palmatinervii* included 33 species and two varieties of palmately veined herbs, shrubs, or small trees. His concept of the genus remained the same for his treatment of *Senecio* in Standley (1926), although there were several additions and deletions of the species that he ascribed to the two sections.

Tangled within the attempt to separate sections and/or genera from *Senecio* s.l. is the concept of cacalioid and senecionoid complexes, first defined by Cassini (1827). His cacalioid complex included those plants with white- or cream-colored disk florets, eradiate capitula, and elongate corolla lobes, and his senecionoid complex had yellow corollas, typically radiate capitula, and short corolla lobes. These complexes have met with varying degrees of acceptance as natural groupings.

Over time, several philosophical trends developed. In 1837, de Candolle summarized Cassini and other post-Linnaean authors, revising the cacalioid complex to include species occurring in Asia, eastern North America, and Mexico that differed consistently from *Senecio* in a number of respects, treating it as the single widespread genus *Cacalia* L. Botanists who have accepted this circumscription are Gray (1883), Hoffman (1894), Greenman (1901), Fernald (1950), Blake (1960), and Gleason and Cronquist (1963). Many authors did not accept the de Candollean *Cacalia*, preferring to relegate all of the species to *Senecio*: Schultz (1845), Bentham (1873a, b), Hemsley (1881), McVaugh (1972, 1984), Rzedowski (1978), and Barkley (1985a, b). Concurrently, in the 20th century, some authors accepted the concept of splitting *Senecio* s.l. into cacalioid and senecionoid complexes, and then within these complexes creating segregate genera. These authors include Rydberg (1924), Cuatrecasas (1960, 1981), Pippen (1968), Robinson and Brettell (1973a, b, c, 1974), and Nordenstam (1978). In 1984, Jeffrey and Chen were the first to recognize the two complexes as subtribes: the senecionoid complex as the subtribe Senecioninae and the cacalioid complex as the subtribe Tussilagininae. Bremer (1994) used this concept, defining the two subtribes primarily on three characters. Species in the Senecioninae have balusterform filament collars, style branches with separate stigmatic areas, and a basic chromosome number of $x = 10$ or 5. Species in the Tussilagininae have cylindrical filament collars, style branches with a continuous stigmatic area, and a basic chromosome number of $x = 30$.

As for *Roldana*, in 1924 Rydberg revised three genera distributed in North and Central America out of the cacalioid and senecionoid complexes. In circumscribing these genera, he pointed out that the characters by which *Cacalia* was being separated from *Senecio* were inconsistent. Rydberg noted that some "true" senecios have cream-colored disk corollas, many are rayless, and that a few species of *Cacalia* have short corolla lobes. As he so aptly stated: "The trouble has arisen because botanists have included in *Cacalia* several different types which can easily be distinguished from each other and from *Senecio* if considered separate but not if taken as an aggregate" (Rydberg, 1924: 369). It is on this foundation that he revised the three genera *Psacalium*, *Odontotrichum* Zucc., and *Pericalia*. For the purposes of this paper, only the genus *Pericalia* is relevant. *Pericalia* is circumscribed as having four species (*P. sessilifolia* (Hook. & Arn.) Rydb., *P. suffulta* (Greenm.) Rydb., *P. ovatifolia* (Sch. Bip.) Rydb., and *P. michoacana* (B. L. Rob.) Rydb.) with the following suite of characters: short corolla lobes, the style branches of *Senecio*, and corollas white or nearly so (although, in fact, the corollas may be orange in *P. suffulta*).

Pippen defined cacalioid and senecionoid characteristics (1968: 371) and circumscribed species based on the combination of these and other characters. Following Rydberg, he expressed the opinion that: "The distinctive elements of North America and Asia formerly placed in *Cacalia* would be better placed in several narrowly circumscribed segregate genera." He considered the section *Palmatinervii* as cacalioid and related to *Psacalium*, *Senecio angulifolius* DC., and *S. cooperi* Greenm. and recognized four segregate genera from the Mexican cacalioid complex: *Digitacalia*, *Odontotrichum*, *Pericalia*, and *Psacalium*. Without explanation, he only included *P. sessilifolia* and *P. michoacana* in the genus *Pericalia*, yet he was aware of Rydberg's paper and cited it.

The first monograph of the section *Palmatinervii* was completed by Gibson in 1969. While never published, it is a reservoir of information. Gibson included 27 species and five varieties in the section. He was the first to pull several species from the section *Fruticosi* and the genus *Pericalia* under the fabric of *Palmatinervii*. While all of the species were placed in the genus *Senecio*, he considered most species of *Palmatinervii* as senecionoid in character, with other species alternately categorized as senecionoid, cacalioid, or as occupying a confusing intermediate group.

Robinson and Brettell (1974) were the first to recognize the section as a distinct genus under the resurrected name *Roldana*. They considered it to be a cacalioid entity and grouped it with other genera that are concentrated in Mexico and have single fused stigmatic surfaces on the style branches and unenlarged bases on the filament collars. Giving the genus a broad interpretation, they included the entire section *Palmatinervii*, part of section *Fruticosi*, the genus *Pericalia*, and one problematic species, *Senecio heteroideus* Klatt, from *Digitacalia*. They circumscribed 46 species and one variety within the genus.

Barkley (1985a) retained the concept of *Senecio* s.l. divisible on the basis of microcharacters into senecionoid and cacalioid assemblages, respectively. It was his belief that until revisionary work at the

species level was completed, further generic consideration would not prove meaningful. His "working groups" are fundamentally in accord with the above authors.

In 1996, Barkley, Clark, and Funston outlined the segregate genera of *Senecio* s.l. and *Cacalia* s.l. in Mexico and Central America, placing *Roldana* in the subtribe Tussilagininae. The circumscription of these genera is in agreement with the views of King and Robinson (1970), wherein consistent morphological characters at the generic level were outlined. These characters have been used worldwide to help illuminate the natural relationships within the tribe Senecioneae. Furthermore, it has become apparent that the groupings that authors since Greenman have used are essentially equivalent to genera.

It is against this backdrop that the current revision of *Roldana* is written. Here, the genus is placed in the subtribe Tussilagininae and defined as having 48 species and eight varieties. It closely follows Robinson and Brettell (1974) in that it includes species from section *Palmatinervii*, the genus *Pericalia*, four species from section *Fruticosi*, and *Senecio heteroideus*, as well as 19 taxa culled from botanical literature.

Morphology

Habit and Architecture

The species of *Roldana* fall into three general groups of life forms, even though they are somewhat imprecise and some species are intermediate. The first group is comprised of sparingly branched or acaulescent herbaceous perennials that are mostly less than 1 m in height. The second group includes strict, erect, single-stemmed perennials that may grow to 4 m in height and are herbaceous or weakly ligneous toward the base. These plants are often solitary or loosely clustered in few-stemmed groups. Plants of the third group are shrubby or tree-like with branching stems that are either solitary or in crowded clusters. At least the lower stems are clearly ligneous, and growth rings are present in the small trees. Root structure in the genus consists of a woody, typically lanate caudex with spreading rhizomes. In perennial herbs, the caudex is often bulbous. Field observations in Mexico revealed that vegetative reproduction occurs either by new growth from the caudex, by rhizomatous growth from the caudex, or by adventitious vegetative growth from stems that have fallen. In the latter case, where nodes from fallen stems come in contact with the ground, roots and then shoots develop, expanding the colony of plants.

The important characters relative to the stem are shape, color, and vestiture; stem shape is typically terete, although *Roldana acutangula* (Bertol.) Funston has angulate stems and *R. hartwegii* has both types of stem shape. The bark on all of the tree species is smooth with small fissures and brownish grey in color, and new growth is red or light brown. The stems of the other habit forms are either red or greenish cream. Vestiture is an important diagnostic feature used in the key, varying from glabrous to a tawny lanate tomentum. Species fall into four general categories. The first category includes those plants that are glabrous, or essentially so, and are suffruticose herbs from the Trans-Mexican Volcanic Belt and northward. The second category contains those species with a light to dense covering of stipitate-glandular hairs and multicelled hairs that are found throughout *Roldana*'s distribution. The third type of pubescence has thin white trichomes that are arranged in either a light to dense arachnoid pubescence or a tomentum of short curly or long tawny hairs. Species with this type of pubescence are predominantly found along the Trans-Mexican Volcanic Belt and southward. Lastly, *R. petasitis* (Sims) H. Rob. & Brettell has a unique pubescence of tawny canescent hairs on the undersurface of the leaves, while the pubescence of the capitulescence is stipitate-glandular.

Leaves

The majority of species have cauline leaves that are evenly distributed on the upper half of the stem, the lowermost being deciduous. Many of the glabrous perennial herbs have cauline leaves that are clustered about the middle of the stem, and the low-growing herbs have basally clustered leaves.

Leaf blade venation is pinnate to pinnipalmate, palmate, or triple-nerved. There are nine species with pinnately veined leaves. The rest are distinctly palmately veined or triple-nerved, i.e., there are three main veins arising from the base of the leaf with secondary veins arising from the central main vein. The petiole is typically marginally attached to the blade. *Roldana subpeltata* (Sch. Bip.) H. Rob. & Brettell, *R. cordovensis* (Hemsl.) H. Rob. & Brettell, *R. heterogama* (Benth.) H. Rob. & Brettell, and sometimes *R. angulifolia* (DC.) H. Rob. & Brettell have eccentrically peltate leaves. *Roldana sessilifolia* (Hook. & Arn.) H. Rob. & Brettell may have sessile cauline leaves.

Blade shape is typically ovate, rotund, lanceolate, or elliptic in outline. The blade margin is typically palmately lobed with some secondary lobing, the sinus depth extending one quarter to one half of the way to the midrib. Several species have blades with serrate margins. All species have callous denticles on the margins. The smallest principal cauline leaves in the

genus belong to *Roldana reticulata* (DC.) H. Rob. & Brettell and are 4–8 × 4–8 cm. *Roldana greenmanii* H. Rob. & Brettell and *R. gilgii* (Greenm.) H. Rob. & Brettell have the largest leaves, up to ca. 30 × 30 cm. Leaf pubescence varies from glabrous to a persistent lanate tomentum.

CAPITULESCENCE

The capitulescence in *Roldana* is terminal, except in *R. glinophylla* Gibson ex H. Rob. & Brettell, where it is axillary. Most species have a pyramidal compound paniculate cyme, but there are 10 species that are flat-topped and described as corymbiform. The capitulescences of *R. mexicana* (McVaugh) H. Rob. & Brettell and *R. glinophylla* have an umbel-like appearance. Plants with a simple panicle and 1 to 5 capitula per branch are the small perennial herbs. In an attempt to quantify the density of capitula within the capitulescence, the character number of capitula per branch was recorded. Although it does not aid in the separation of capitulescence types as well as anticipated, it was kept in the descriptions as it is of some diagnostic use.

Ultimate peduncle length is a useful diagnostic character. The typical peduncle length is 2–10 mm. Those plants with distinctly long ultimate peduncles are *Roldana ehrenbergiana* (Klatt) H. Rob. & Brettell and *R. sessilifolia*, which have peduncles exceeding 20 cm, and *R. guadalajarensis* (B. L. Rob.) H. Rob. & Brettell, *R. heteroidea* (Klatt) H. Rob. & Brettell, *R. michoacana* (B. L. Rob.) H. Rob. & Brettell, and *R. suffulta* (Greenm.) H. Rob. & Brettell, which have peduncles ranging from 10–15 cm. *Roldana lobata* La Llave and *R. robinsoniana* (Greenm.) H. Rob. & Brettell have sessile capitula that are described as being arranged in glomerules. Pubescence varies from glabrous to lanate tomentose.

The character primary bracts of the capitulescence refer to those bracts that arise at the level of the secondary peduncles as opposed to the bracts of the rachis. Robinson and Brettell (1974) were the first to use the description of these bracts as a diagnostic character. The bracts have three forms: linear, filiform, and obovate. The typical forms that these bracts take are linear or filiform and are 3–5 mm in length. The species *Roldana angulifolia*, *R. cordovensis*, *R. greenmanii*, *R. grimesii* (B. L. Turner) C. Jeffrey, *R. petasitis*, and, on occasion, *R. sessilifolia* have large obovate bracts that are 5–15 mm in length. With regard to the rachis bracts, *R. greenmanii*, *R. petasitis*, and *R. cordovensis* may have uniquely large obovate bracts. The term bracteole refers to those structures that are found on the ultimate peduncles of the capitulescence. These are typically linear in shape and 1–3 mm in length. *Roldana subpeltata* is ebracteolate. Only *R. angulifolia*, *R. grimesii*, and *R. suffulta* have obovate bracteoles.

CAPITULA

Roldana has radiate and eradiate capitula. They are homochromous; corolla color is yellow, yellow-orange, or cream-white. Capitula are turbinate, campanulate, or funnelform in shape. The average capitula size is ca. 10 × 3 mm. Capitulum height is measured from the point where the capitulum attaches to the peduncle to the top of the mature florets, excluding ray ligules. Width is measured across the base of the capitulum where the phyllaries attach to the receptacle. All measurements were taken from dried herbarium specimens. Calyculate bracts arise from the base of the capitula. Typically they are 1 to 3 in number, linear in shape, and 1–3 mm in length. Calyculate bracts are completely lacking in *R. subpeltata*. Obovate calyculate bracts are found on *R. angulifolia*, *R. grimesii*, *R. reticulata*, and *R. suffulta*. The phyllaries are of uniform length but are arranged in two weakly defined rows, the margins of adjacent phyllaries overlapping. The phyllaries lying toward the inside of the row have hyaline margins. The base of the phyllary is thickened dorsiventrally, a condition representative of the subtribe and best observed on fresh specimens. The one exception in the genus is *R. mixtecana* Panero & Villaseñor, which has phyllaries in five graduated series. When dry, they have a greenish brown appearance and purple striations may be present. The pubescence of the phyllaries typically matches that of the peduncles, although at times it may not, and this difference can aid in distinguishing the species. The number of phyllaries present has traditionally been used as a diagnostic character. Typically, species fall into one of three categories: 5 phyllaries, 6 to 8 phyllaries, and 10 to 14 phyllaries. However, there are exceptions: *R. hartwegii* has collections with the full range of phyllary number and, while *R. scandens* Poveda & Kappelle and *R. schaffneri* (Sch. Bip. ex Klatt) H. Rob. & Brettell typically have 5 phyllaries, many collections have 6-phyllaried capitula as well. The receptacle surface of the capitula is alveolate and ridged on all species of *Roldana*.

FLORETS

The number of ray florets per capitula is typically 3 to 5. There are three types of ray florets: ligulate, inconspicuously ligulate, and tubular. Ligulate ray florets have conspicuous ligules that are typically 5–7 × 2–3 mm. *Roldana ehrenbergiana* has the largest ray

ligules, measuring 10–30 × 5–10 mm. Inconspicuous ray florets have ligules that measure only ca. 2 × 1 mm. Tubular ray florets do not have a ligule, and the tube itself is typically 2–3 mm long. The presence/absence of rays in some species is a variable character. Species that have pubescent ray florets are *R. kerberi* (Greenm.) H. Rob. & Brettell, *R. grimesii*, *R. hartwegii*, *R. hirsuticaulis* (Greenm.) Funston, *R. lobata*, and *R. subcymosa* H. Rob.

The number of disk florets per capitulum is typically less than 30. *Roldana ehrenbergiana*, *R. sessilifolia*, and *R. suffulta* have large capitula with 90+ disk florets. The shape of the corolla is typically funnelform, although a few species are distinctly campanulate. The corolla is divided into tube, throat, and lobes (see Pippen, 1968). All species have 5 acute lobes. Species in *Roldana* have the senecionoid type of lobe length, that is, it is shorter than or equal to the throat, with one exception, *R. eriophylla* (Greenm.) H. Rob. & Brettell, which has lobes longer than the throat. Species with a pubescent disk corolla are *R. kerberi*, *R. grimesii*, *R. hartwegii*, *R. hirsuticaulis*, *R. lobata*, *R. subcymosa*, and *R. subpeltata*. I have found the presence or absence of ray and disk corolla pubescence to be a reliable character.

ACHENES

The achenes within *Roldana* are typically 1–2 mm long and cylindrical in shape, and have 5 or 10 vertical ribs. Nine species have pubescent achenes, and *R. barba-johannis* (DC.) H. Rob. & Brettell and *R. cordovensis* have achenes with distinct resin glands. The pappus is of numerous capillary bristles and is in several series. It is typically ca. 1 mm shorter than the total length of the disk corolla.

CHROMOSOMES

Relatively few species of *Roldana* have had chromosome counts made. For those that have been examined, all are reported to be $n = 30$ (Turner et al., 1962; Ornduff, 1963; Turner & King, 1964; Turner & Flyr, 1966).

PHYTOGEOGRAPHY

There are two centers of diversity in *Roldana*: one is along the Trans-Mexican Volcanic Belt (TVB, Eje Neovolcánico) and the Sierra Madre del Sur, the second is along the Sierra Madre de Chiapas and Guatemala. The 48 species can be grouped into six putative clades, subjectively based on a combination of morphological characters and, to a lesser extent, distribution. The species of *Roldana* are found predominantly in the pine-oak forests of Mexico and Guatemala, and it is through their phytogeography that clues to *Roldana*'s dispersal are derived.

Roldana's distribution spans eight countries, from the extreme southern parts of Arizona and New Mexico in the United States to the southernmost end of the Cordillera de Talamanca in Panama. Composed of weedy species, it grows in disturbed areas and in the understory of pine-oak forests throughout the highlands of Mexico and Central America. It typically grows at elevations between 2000 and 4000 m, although it has been collected at elevations as low as 900 m and as high as 4100 m. Mexico is comprised of 15 physiographic regions; *Roldana* occurs within eight of these regions. Appendix 1 provides a species list for each region. Fifty-six percent of the species in *Roldana* occur along the TVB with four regional endemics. The Sierra Madre del Sur also contains 56% of the species and has six regional endemics. The Sierra Madre Oriental has 38% of the species and three endemics, the majority being restricted to the extreme southern end of the mountain chain, which runs into the eastern end of the TVB. The Sierra Madre Occidental has 23% of the species, including three endemics, with the species evenly dispersed along the mountain chain. Another significant area of diversity for *Roldana* lies below the Isthmus of Tehuantepec in Mexico southward to the Cordillera de Talamanca in Panama, where there are 14 species equaling 29% of the genus. While the species in this region have a distinct aspect to them, there is little doubt that they should be included within *Roldana*. *Roldana acutangula*, *R. gilgii*, *R. greenmanii*, and *R. scandens* (endemic to Costa Rica) do not occur above the Isthmus of Tehuantepec. All of the other Central American species are concentrated in the Guatemalan highlands, yet may reach as far north as the Sierra Madre Oriental and as far south as the aforementioned Cordillera de Talamanca in Panama (Appendix 2). In contrast, *R. aschenborniana* (S. Schauer) H. Rob. & Brettell and *R. barba-johannis* are the only two species with distributions that are concentrated in the TVB and Sierra Madre del Sur that extend south of the Isthmus of Tehuantepec. The patterns of concentration in the regions of Mexico and Central America are similar to those found in *Senecio* s.l. by Barkley (1990) and that of the generic distribution in Mexico of the Senecioneae by Turner and Nesom (1993).

To further elucidate the distributional patterns within the genus, I grouped the species of *Roldana* into six putative clades primarily based on similar habit and morphology (Appendix 3). The Central American complex is predominantly made up of woody shrubs and small trees that have a stipitate-glandular pubescence that includes multicelled

trichomes. The species are distributed in the highlands of Guatemala and Central America, with two notable exceptions. *Roldana hintonii* H. Rob. & Brettell and *R. mezquitalana* (B. L. Turner) Funston occur in the TVB and the Sierra Madre Occidental, respectively, but were placed here due to their strong resemblance to *R. schaffneri*. Further data (e.g., DNA) showing the relationships among these three species would give clues to the overall dispersal history of the genus. The *R. hartwegii* complex is composed of herbs and subshrubs with an arachnoid floccose pubescence on the peduncles and a light tomentum on the undersurface of the leaves. They are typically found along the TVB and northward along both the Sierra Madre Occidental and Oriental. The *R. lobata* complex is made up predominantly of woody shrubs and small trees that have a persistent tomentum, which may be tawny on the new growth, and a dense lanate tomentum on the undersurfaces of the leaves. *Roldana gonzaleziae* (B. L. Turner) B. L. Turner and *R. sundbergii* (B. L. Turner) B. L. Turner are perennial herbs, but were placed here due to their tawny pubescence. The *R. lobata* complex is centrally concentrated about the TVB and Sierra Madre del Sur but also extends northward along the Sierra Madre Oriental and Occidental as well as southward into Central America. The species *R. petasitis* with its three varieties was placed in a complex unto itself due to its broad distribution and mixed pubescence of woolly hairs on the undersurface of the leaves and stipitate-glandular hairs on the peduncles and phyllaries. Its core distribution is in the highlands of Guatemala. At its northernmost extension, much of its unique pubescence is lost, and *R. petasitis* appears to hybridize with *R. angulifolia* in the southern ranges of the Sierra Madre del Sur. The *R. cordovensis* complex is made up of suffruticose shrubs that have a stipitate-glandular pubescence. It is like the *R. hartwegii* complex in that it is distributed throughout the TVB and northward along the Sierra Madre Oriental. Lastly, the *R. pericalia* complex includes all of those species formerly placed in the genus *Pericalia* by Rydberg (1924), which included eradiate glabrous suffruticose herbs to subshrubs. I have extended that definition to include radiate species and plants that are essentially glabrous. This group, like the *R. lobata* complex, is centered in the TVB with some northern and southern extensions.

Examination of the clades reveals that there are two areas of diversity in the genus. The Central American and *Roldana petasitis* complexes are concentrated about the Guatemalan highlands and rarely reach above the Isthmus of Tehuantepec. The majority of species in the other four clades are concentrated throughout the TVB and Sierra Madre del Sur with northern extensions along both the Sierra Madre Occidental and Oriental.

What is the explanation for this distribution? Were the progenitors of *Roldana* from North America or South America? One theory is that *Roldana* is a descendant of the senecios of Eurasia, which were dispersed to North America via the arcto-tertiary geoflora of classical phytogeography (T. M. Barkley, pers. comm.). Another possibility is that they dispersed from South America across the Panamanian land bridge. The difficulty with the latter theory is that the tropical flora of Mexico are typically found below ca. 1000 m (3500 ft., Miranda & Sharp, 1950). Miranda and Sharp (1950) also note that the transition zone between 1000 m and 1200 m (3300–4000 ft.) is the dividing line between the arcto-tertiary and Neotropical floristic regions. Graham (1973, 1975, 1976) and Gentry (1982) give further evidence that, while the lowland tropical plants of Central America and southern Mexico have dispersed northward from South America, this does not include plants above the elevation of the tropical flora. *Roldana* makes its home in the pine-oak forests above ca. 1500 m, and these forests have traditionally been allied with the flora of North America. Even in the regions of Central America where *Roldana* is found, the habitat has more affinities with the Mexican flora than that of South America (Steyermark, 1950; Graham, 1993). In particular, Steyermark remarks that the pine-oak forests of the Cuchumatanes and Sierra de las Minas of Guatemala resemble the southern Rocky Mountains, especially those of New Mexico. Therefore, I concur with Barkley that the most likely avenue of dispersal is from North America.

The geo-histories of Mexico and Central America give clues to the distribution patterns of senecios into Mexico. Barkley (1990) believes that *Senecio* s.l. or its progenitors diversified into these regions during the Late Tertiary. From the Cretaceous through the Quaternary, these regions underwent extensive tectonic activity (Coney, 1982). The Sierra Madre Occidental is believed to have arisen during the Cretaceous and Paleocene. The TVB was developed during the end of the Tertiary, with its principal uplift from the Miocene to the Quaternary. By the end of the Tertiary, southern Mexico had access to temperate biotas of North America and tropical biotas to the south (Graham, 1993). These invasions were precipitated by warm-cool and wet-dry climatic fluctuations that varied independently of each other. It is believed that the areas of temperate biotas increased during the cool climates of the Late Eocene, Middle Miocene, and the Pleistocene, while the tropical biotas increased during the warm climates of the Paleocene, Early and Middle Eocene, Oligocene, and Early

Miocene (Toledo, 1982; Graham, 1993). The ebb and flow of these biotas created cycles of refugia and primary communities that surely affected speciation rates through vicariance.

McDonald (1993) provides indirect geological and paleological evidence that suggests that montane vegetation in northeastern Mexico and adjacent regions descended ca. 1000 to 1300 m below their present-day positions during the peak of the Wisconsin glacial (70,000–12,000 years before present). This lowering would have provided migration corridors for montane elements of Mexico during colder periods. As the montane vegetation returned to higher elevations, it would cut off these routes, isolating populations and creating refugia. His theory holds that continuous montane forests would have occurred all along the Sierra Madre Occidental and would have been intermittent along the TVB and Sierra Madre del Sur. These events recurred, creating short-term isolated populations followed by the re-integration of populations.

Perhaps the most cogent explanation of the factors affecting the dispersal and speciation events within montane flowering plants is given by Takhtajan (1968). Montane regions form pockets of disjunct populations in which genetic drift occurs. Genetic drift enhances the isolation of forms that are statistically unlikely to be expressed in larger populations due to natural selection. The accumulation of small neutral mutations in these isolates is greater than that of larger populations. Polymorphisms or new adaptations in intraspecific forms may arise, which may ultimately play a role in interspecific differences. *Roldana hartwegii*, with its variation in phyllary number and achene pubescence, may well be an example of this phenomenon. Occasional interbreeding between such populations increases their variability and, hence, their plasticity. Hybridization produces more material suitable for rapid adaptive evolution, thus creating evolutionary jumps without evidence of precursor organisms. The endemics found in the TVB, Sierra Madre del Sur, and both the Sierra Madre Occidental and Oriental may be examples of this type of speciation event.

An important piece that is missing from the puzzle is the reproductive biology of the genus. Unfortunately, the breeding mechanisms of *Roldana* are a matter of complete speculation. Barkley (1990) believes that they function similarly to the aureoids of North America, in that they are normally outcrossers with generalized pollinators and that their cytological structure allows easy hybridization and introgression. Because the chromosome number is $n = 30$ in a tribe in which the base number is thought to be $x = 10$, the ancestor of the genus (and probably all of the tussilaginoids) was hexaploid, although the descendants are now functioning as diploids. It should also be noted that many species are prodigious vegetative reproducers. All signs point to the fact that *Roldana* is a genus that is comfortable in disturbed habitats. In this regard, the role of man cannot be ignored. Since ancient times, the greatest part of the Mexican populace has inhabited the pine-oak forests of the TVB and Sierra Madre del Sur. Irrigated agriculture has been carried on there for some 6000 years (Barkley, 1990). Bye (1993) discusses the interplay between mankind and the diversification of weeds in the Mexican flora. For millennia, the people of the Tehuacán Valley have exploited the biotic community for medicine and food, creating disturbed habitats throughout the region that are favorable to the proliferation of weedy species.

In conclusion, the species of *Roldana* probably have breeding mechanisms that allow for hybridization and introgression when populations come in contact with each other. The fluctuating climate and high level of tectonic activity of Mexico and Central America since at least the Early Tertiary have provided an ever-changing montane environment. This varying habitat caused a recurring cycle of refugia and primary communities within the pine-oak forests with a concomitant cycle of short-term isolation of breeding populations in *Roldana* followed by a re-integration of those populations. The net effect of these cycles, and recent human habitation, is a morphologically variable genus resulting from the evolutionary processes of natural selection and genetic drift. As with all genera, details concerning the phytogeography and evolution await a well-resolved phylogeny of the species.

Taxonomic Treatment

Roldana La Llave, Nov. Veg. Descr. 2: 10. 1825. TYPE: *Roldana lobata* La Llave. *Senecio* sect. *Palmatinervii* Hoffm. in A. Engler & K. Prantl. 1894. Die Naturlichen Pflanzenfamilien. Leipzig. 4: 64.

Perennial herbs, suffruticose shrubs, and small trees, 0.2–8.0 m tall, arising from a fibrous-rooted, woody, often lanate caudex, growing singly or in colonies; stems erect to ascending, typically not foreshortened below capitulescence, glabrous to having a tawny lanate tomentum. *Leaves* simple, alternate, venation palmate to pinnate; petiolate to rarely peltate or sessile, cauline, variously clustered to ± evenly distributed on upper half of stem; ovate, orbicular, lanceolate, elliptic, or pinnatifid, typically 5- to 13-lobed, lobes acute or rounded, sinus depth typically about 1/2 of the way to the midrib but ranging from subentire to dissected, abaxial surface

glabrate to densely lanate tomentose and canescent, adaxial surface typically glabrescent, margins variously callous denticulate. *Capitulescence* typically a compound paniculate cyme, rarely a corymbiform cyme, or a simple panicle; ultimate peduncles typically 6–10 mm, but reaching up to 22 cm in some species; rachis bracts typically minutely foliaceous, or obovate; primary bracts and bracteoles may be filiform, linear, or obovate. *Capitula* radiate or eradiate; homochromous, corolla cream, yellow, or orange (may appear white when dry); capitula 7–10(25) × ca. 3(10) mm; calyculate bracts ca. 5, filiform, linear, or obovate; phyllaries 5 to 14(25), typically in 1 apparent series, margins overlapping, or graduated in 5 series, typically glabrous or stipitate-pubescent, but may be arachnoid or tomentose, ca. 8(14) × 1–3 mm. Receptacle surface alveolate and ridged. Ray florets absent or 3 to 5(8), corollas ligulate, inconspicuous (ligule measuring less than 3 mm), or reduced to only a tube, glabrous or pubescent, ligule 5–7(30) × ca. 3(10) mm; venation typically 4, unbranched. Disk florets 6 to 30(90+), corolla funnelform or campanulate, 5-lobed, glabrous or pubescent, typically 8–10 mm, lobes of the corolla as long as or shorter than the throat. Stigmatic surface entire, style branch apex conical without apical tufts. Stylopodium free, style base cylindrical or expanded. Filament collar cylindrical. Anther base rounded or short-tailed, anther appendage about twice as long as wide or slightly longer. Endothecial tissue semi-polarized. Achenes glabrous or pubescent, cylindrical, 1–2 mm, ribs 5 or 10, resin glands typically absent; pappus of numerous white capillary bristles, in several series, ca. 7–9 mm. Pappus hairs tapered (rarely clavate), 2 or 3 apical cells divided (rarely appressed). Carpopodium medium or large. Ovary wall crystals mixed, predominantly druses or quadrates. Achene glabrous, or pubescent with short clavate or long thin hairs.

Distribution and ecology. Extreme southern Arizona and New Mexico in the United States, Mexico, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, and Panama at elevations of 1000–4100 m. Montane cloud forests and pine-oak forests. Distributed in understory, margins of open grassland, waste places, and along roadsides. Associated with *Abies* Mill., *Alnus* Mill., *Arbutus* L., *Baccharis* L., *Chiranthodendron* Larreat., *Clethra* L., *Colubrina* Rich. ex Brogn., *Cornus* L., *Cupressus* L., *Dahlia* Cav., *Distylium* Siebold & Zucc., *Eryngium* L., *Ficus* L., *Fraxinus* L., *Fuchsia* L., *Ipomoea* L., *Liquidambar* L., *Meliosma* Blume, *Mimosa* L., *Ostrya* Scop., *Penstemon* Schmidel, *Physalis* L., *Phytolacca* L., *Pinus* L., *Platanus* L., *Podocarpus* Labill., *Populus* L., *Potentilla* L., *Prunus* L., *Quercus* L., *Salvia* L., *Senecio*, *Solanum* L., and *Symphoricarpus* Kunth. Flowering time is year round, typically November through April.

Key to the Species of *Roldana* in the Southwestern United States, Mexico, and Central America

1a. Leaf blade venation pinnate (to pinnipalmate as in *R. barba-johannis* and *R. neogibsonii*).
  2a. Phyllaries 5 to 8.
    3a. Leaf blades elliptic-linear, margins entire to irregularly serrate; phyllaries 5 or 6 . . . . . 43. *R. schaffneri*
    3b. Leaf blades pinnately 7- to 11-lobed; phyllaries 7 or 8.
      4a. Achenes pubescent; leaf blade ca. 7-lobed with sinus depth more than 1/2 of the way to the midrib, base decurrent on the petiole forming a wing, abaxial surface glabrescent . . . . . 20. *R. heracleifolia*
      4b. Achenes glabrous; leaf blade 7- to 11-lobed with sinus depth typically no more than 1/3 of the way to the midrib, blade not decurrent on petiole, abaxial surface densely pubescent to lanate tomentose . . . . . 29. *R. lineolata*
  2b. Phyllaries 9 to 14.
    5a. Plants perennial herbs; capitula with 50 to 60 disk florets . . . . . 9. *R. ehrenbergiana*
    5b. Plants suffruticose herbs to shrubs; capitula with 9 to 20 disk florets.
      6a. Peduncles and abaxial leaf blade surface persistently tomentose.
        7a. Peduncles and abaxial leaf blade surface velvety tomentose; leaf blades oblong . . . . . 36. *R. neogibsonii*
        7b. Peduncles and abaxial leaf blade surface tomentose with white woolly hairs; leaf blades broadly lanceolate, oval, or ovate . . . . . 7. *R. barba-johannis*
      6b. Peduncles and abaxial leaf blade surface glabrescent, stipitate-pubescent, or arachnoid pubescent.
        8a. Plants essentially glabrous; leaf blades linear, 7–10× longer than wide . . . . . 17. *R. guadalajarensis*
        8b. Plants pubescent; leaf blades elliptic, ca. 3× longer than wide.
          9a. Capitulescence pubescent with appressed arachnoid hairs; leaf margins entire to subentire; known only from Temascaltepec, México . . . . . 23. *R. hintonii*
          9b. Capitulescence densely stipitate-pubescent; leaf margins serrulate; known only from the states of Durango and Jalisco . . . . . 33. *R. mezquitalana*
1b. Leaf blade venation palmate- to triple-nerved.
  10a. Phyllaries 25 to 35, in five graduated series . . . . . 35. *R. mixtecana*
  10b. Phyllaries 5 to 14, in one apparent series.

11a. Phyllaries 5 to 8.
12a. Plants essentially glabrous.
13a. Leaf blades deeply palmately lobed to dissected; achenes glabrous . . . . . 22. *R. heteroidea*
13b. Leaf blades ovate to orbicular, margins serrate to lobed with sinus depth less than 1/2 of the way to the midrib; achenes pubescent.
14a. Leaf blades with petioles marginally attached; corollas glabrous . . . . . 32. *R. mexicana*
14b. Leaf blades eccentrically peltate; corollas pubescent . . . . . 45. *R. subpeltata*
12b. Plants variously pubescent.
15a. Plants acaulescent perennial herbs, typically 20–70 cm tall.
16a. Achenes glabrous; corollas pubescent; found in Durango and Jalisco . . 14. *R. gonzaleziae*
16b. Achenes pubescent; corollas glabrous; found in Nuevo León . . . . . 47. *R. sundbergii*
15b. Plants caulescent perennial-suffruticose herbs, shrubs, or trees, typically greater than 1 m high.
17a. Plants with a primary pubescence type of stipitate-glandular and/or multicelled hairs; peduncles always variously stipitate-pubescent, abaxial leaf blade surface glabrous to densely and persistently pubescent with stipitate-glandular and/or multicelled hairs or variously tomentose (as in *R. petasitis*).
18a. Cauline leaves variously hastate to having 3 divaricate lobes, secondarily lobed to merely denticulate.
19a. Capitula radiate; ray ligule 10–13 × 2–4 mm; from Oaxaca . . 5. *R. anisophylla*
19b. Capitula eradiate or radiate with ray ligule inconspicuous.
20a. Capitula eradiate; from Oaxaca . . . . . 19. *R. hederifolia*
20b. Capitula eradiate or rays 3 to 5, ray ligule ca. 3 × 0.5 mm; from México and Michoacán . . . . . 3. *R. aliena*
18b. Cauline leaves variously palmatifid, orbicular, ovate, or oblong; 5- to 7(+)-lobed.
21a. Leaf blades orbicular to ovate, typically 7(+)-lobed.
22a. Leaves eccentrically peltate; cauline leaves palmately (5 to)7- to 17-lobed, lobes acute . . . . . 21. *R. heterogama*
22b. Leaf petioles marginally attached; cauline leaves ovate to orbicular, 7- to 13-lobed.
23a. Leaf lobes 9 to 13, deeply cut forming rectangular segments topped with 3 acute lobes; blades abaxially pubescent with short and long multicelled hairs, densely so on veins . . 15. *R. greenmanii*
23b. Leaf lobes ca. 7, shallow; blades abaxially pubescent to densely lanate tomentose . . . . . 37. *R. petasitis*
21b. Leaf blades variously palmatifid, ovate to oblong, 5- to 7(13)-lobed.
24a. Capitulescence a simple cymose panicle; leaves clustered at the base or midstem . . . . . 31. *R. metepeca*
24b. Capitulescence a compound paniculate cyme; leaves cauline, evenly distributed on stem, lowermost deciduous.
25a. Cauline leaves distinctly secondarily lobed; calyculate bracts ca. 3, obovate, 5–15 × ca. 2 mm; phyllaries 9–13 × 1.3–2 mm . . . . . 4. *R. angulifolia*
25b. Cauline leaves without secondary lobing or minutely so; calyculate bracts 0 to 3, linear to filiform, 1–2 mm; phyllaries 7–9 × ca. 1 mm.
26a. Cauline leaves eccentrically peltate . . . . . 8. *R. cordovensis*
26b. Cauline leaf petioles marginally attached.
27a. Cauline leaves ovate to orbicular, shallowly ca. 7-lobed; adaxially puberulent, abaxially pubescent to densely lanate tomentose . . . . . 37. *R. petasitis*
27b. Cauline leaves palmatifid, 5- to 7-lobed; adaxially glabrescent, abaxially glabrescent to stipitate-glandular pubescent.
28a. Capitula 12–15 × 3–5 mm; from northern Mexico (Chihuahua, Durango, Sinaloa) . . . . . 11. *R. gentryi*
28b. Capitula 6–11 × 2–3 mm; from southern Mexico (Chiapas, Oaxaca, Veracruz) and Central America.
29a. Primary bracts linear, 2–4 mm long; ultimate peduncles ca. 6 mm long; bracteoles 0 to 3, linear to scale-like, 1–2 mm long . . . . . 25. *R. jurgensenii*
29b. Primary bracts linear, 5–15 mm long or obovate 10–30 × 5–15 mm; ultimate peduncles 8–20 mm long; bracteoles 0 to 3, filiform, up to 3 mm long . . . . . 37. *R. petasitis*

17b. Plants with a primary pubescence type of arachnoid or woolly hairs; peduncles and abaxial leaf blade surfaces variously glabrate, arachnoid, or tomentose pubescent.
30a. Peduncles and abaxial leaf blade surface variously tomentose pubescent.
31a. Leaves seasonally deciduous, new leaves emanating from stem apex, creating a foreshortened stem . . . . . 10. *R. eriophylla*
31b. Leaves cauline about evenly distributed on upper half of stem, lowermost deciduous, stem not foreshortened.
32a. Plants with a light tomentum on peduncles and abaxial leaf surface, becoming glabrate with age.
33a. Shrubs and small trees, 3–7 m tall . . . . . 2. *R. albonervia*
33b. Single-stemmed perennial herbs, 1–2 m tall . . . . 18. *R. hartwegii*
32b. Plants with a dense persistent, at times lanate, tomentum on peduncles and abaxial leaf surface, never glabrate.
34a. Leaf blades palmately veined, rotund to cordate-reniform, crenate to shallowly 9(+)-lobed; some margins with elongate denticles up to 3 mm long . . . . . 28. *R. lanicaulis*
34b. Leaf blades triple-nerved to palmately veined, ovate to cordate, entire to shallowly ca. 5-lobed; margins never with elongate denticles . . . . . 41. *R. robinsoniana*
30b. Peduncles and abaxial leaf blade surface arachnoid floccose to glabrescent.
35a. Leaf blades distinctly palmately nerved, orbicular . . . . . 42. *R. scandens*
35b. Leaf blades triple-nerved, variously palmatifid or ovate.
36a. Leaf blades palmatifid; plants of montane cloud forests in Chiapas, Mexico, and Guatemala; phyllaries 8, glabrous, 6–7 × 1–2 mm . . . . . 1. *R. acutangula*
36b. Leaf blades variously ovate; plants of pine-oak forests in the southwestern U.S.A. and Mexico; phyllaries (5 to)8 to 13, glabrous to arachnoid floccose, 4–5.5 × ca. 1 mm.
37a. Plants shrubs and small trees, 3–7 m tall . . . . . 2. *R. albonervia*
37b. Plants single-stemmed suffruticose herbs, 1–4 m tall.
38a. Plants 1.5–4.7 m tall, principal cauline leaf blades (5–)10–35 × (2–)10–25 cm, petioles (2–)10–20 cm long . . . . . 26. *R. kerberi*
38b. Plants 1–3 m tall, principal cauline leaf blades 5–14 × 5–16 cm, petioles 3–7 cm long.
39a. Plants glabrate to weakly pubescent with hirsute hairs; rare, known from one collection in Hidalgo . . . . . 39. *R. reglensis*
39b. Plants glabrate to arachnoid floccose pubescent; common . . . . . 18. *R. hartwegii*

11b. Phyllaries 9 to 14.
40a. Plants essentially glabrous.
41a. Capitula radiate.
42a. Leaf blades palmatifid, 5-lobed, distinctly star-shaped, margins entire . . 13. *R. glinophylla*
42b. Leaf blades ovate, margins variously serrate . . . . . 40. *R. reticulata*
41b. Capitula eradiate.
43a. Calyculate bracts oval-elliptic; achenes pubescent . . . . . 46. *R. suffulta*
43b. Calyculate bracts linear; achenes glabrous.
44a. Capitula with 36 to 58 florets; leaf blades never sessile, subpalmatifid, 3- to 5(7)-lobed . . . . . 34. *R. michoacana*
44b. Capitula with 40+ florets; leaf blades may be sessile, subpalmatifid to ovate-elliptic, 5- to 9-lobed . . . . . 44. *R. sessilifolia*
40b. Plants variously pubescent.
45a. Plants acaulescent to subcaulescent perennial herbs, typically 20–70 cm tall.
46a. Petioles 1.5–7 cm long; leaf blades 3.5–7 × 4–7 cm, ovate, with ca. 6 shallow acute lobes; plants glabrous to floccose arachnoid . . . . . 48. *R. tlacotepecana*
46b. Petioles 6–12 cm long; leaf blades 6–11 × 8–13 cm, palmatifid, 5- to 7-lobed, lobes acute, secondarily lobed, sinus depth about 1/2 of the way to the midrib; plants pubescent with a mixture of stipitate-glandular and long multicelled hairs . . . . . 38. *R. platanifolia*
45b. Plants caulescent suffruticose perennial herbs, shrubs, or trees, typically greater than 1 m tall.
47a. Plants with a primary pubescence type of stipitate-glandular and multicelled hairs; peduncles and abaxial blade surface slightly to densely pubescent (sometimes so dense as to appear tomentose in *R. gilgii* and *R. grimesii*).
48a. Phyllaries ca. 1 mm wide.
49a. Leaf blades eccentrically peltate; capitula eradiate . . . . . 21. *R. heterogama*
49b. Leaf blades marginally attached to petiole; capitula radiate . . 27. *R. langlassei*

48b. Phyllaries greater than 2 mm wide.
50a. Capitulescence a simple cymose panicle; leaves clustered at the base or midstem . . . . . 31. *R. metepeca*
50b. Capitulescence a compound cymose panicle; leaves evenly distributed on upper half of stem, lowermost deciduous.
51a. Leaf blades triple-nerved, palmatifid, 5- to 7(13)-lobed, sinus depth 1/2 to less than 1/2 of the way to the midrib; petioles glabrate, marginally attached to blade or eccentrically peltate . . . 4. *R. angulifolia*
51b. Leaf blades palmately veined (to weakly triple-nerved), rotund to reniform, 10(+)-lobed, sinus depth less than 1/4 of the way to the midrib; petioles densely pubescent, marginally attached to blade.
52a. Calyculate bracts 1 to 3, linear, 2–3 mm long, densely pubescent with stipitate-glandular and long multicelled hairs, margins pubescent wih short multicelled hairs, not ciliate . . . . . 12. *R. gilgii*
52b. Calyculate bracts 3 to 6, obovate to elliptic, 10–14 mm long, pubescent with stipitate-glandular hairs, margins ciliate with long multicelled hairs . . . . . 16. *R. grimesii*

47b. Plants with a primary pubescence type of arachnoid or woolly hairs; peduncles and abaxial leaf blade surface glabrate to tomentose.
53a. Peduncles and abaxial leaf blade surface variously tomentose pubescent.
54a. Pubescence of capitula restricted to tomentose tufts at base . . 40. *R. reticulata*
54b. Pubescence of capitula not restricted to base.
55a. Corollas pubescent.
56a. Leaf blades ovate to cordate, with ca. 10 shallow lobes to crenate; petioles and stems lanate tomentose . . 24. *R. hirsuticaulis*
56b. Leaf blades ovate to oval-subpalmatifid, 5- to 7-lobed, sinus depth 1/4 or less of the way to the midrib, lobes acute, or occasionally rotund to oval with subentire margins; petioles and stems floccose tomentose . . . . . 30. *R. lobata*
55b. Corollas glabrous.
57a. Leaf blades ovate, oblong, or rotund, mostly 5- to 9-lobed, sinus depth less than 1/4 of the way to the midrib or margins sinuate; peduncles floccose arachnoid . . . . 6. *R. aschenborniana*
57b. Leaf blades cordate-orbicular, margins crenate to shallowly 9(+)-lobed, sinus depth less than 1/8 of the way to the midrib; peduncles lanate tomentose . . . . . 28. *R. lanicaulis*
53b. Peduncles and abaxial leaf blade surface variously arachnoid pubescent.
58a. Leaf blades oval and subentire to ovate and weakly (3 to)5- to 7-lobed, abaxial surface arachnoid to lightly tomentose pubescent . . . . . 30. *R. lobata*
58b. Leaf blades ovate to oval, (5 to)7- to 15-lobed, abaxial surface glabrate to weakly arachnoid pubescent.
59a. Plants 1.5–4.7 m tall; petioles (2–)10–20 cm long; principal cauline leaf blades (5–)10–35 × (2–)10–25 cm; found along the Sierra Madre de Manantlán and Sierra Madre del Sur . . . . . 26. *R. kerberi*
59b. Plants 1–3 m tall; petioles 3–7 cm long; principal cauline leaf blade 8–14 × 8–16 cm; found throughout the Sierra Madre Occidental and northern Sierra Madre Oriental . . . . . 18. *R. hartwegii*

1. Roldana acutangula (Bertol.) Funston, comb. nov. Basionym: *Cineraria acutangula* Bertol., Novi Comment. Acad. Sci. Inst. Bononiensis 4: 435. 1840. *Senecio acutangulus* (Bertol.) Hemsl., Biol. Cent.-Amer., Bot. 2: 235. 1881. *Roldana acutangula* (Hemsl.) H. Rob. & Brettell, Phytologia 27: 415. 1974, nom. illeg. TYPE: Guatemala. Sacatepéquez: slopes of Volcán de Agua, 1837, *J. Velasquez s.n.* (holotype, BOLO not seen, microfiche IDC 748. 46 III 7!; isotype, BOLO fragm. US not seen).

Robust suffruticose herbs or shrubs, 1–4 m tall; new growth glabrate to arachnoid floccose; stems 4- to 6-angulate, red or brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 8–18 cm, glabrate to sparsely pubescent; blades 12–24 × 6–16 cm, papyraceous, triple-nerved, palmatifid, 5- to 9-lobed, sinus depth 1/2 of the way to less than 1/2 of the way to the midrib, lobes acute, irregularly serrate, margins with multicellular hairs; abaxially glabrate with pubescence of short and long glandular hairs on veins, adaxially glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* compound paniculate cyme (sometimes with a flat-topped corymbiform aspect), 20 to 50 capitula per branch, peduncles arachnoid pubescent to glabrate, primary bracts linear, 1–3 mm, ultimate peduncles 2–7 mm, bracteoles 1 or 2, scale-like to linear, 1–2 mm. *Capitula* radiate, 8–10 × 2–3 mm, funnelform, corollas

yellow; calyculate bracts 0 to 5, linear, 1–2 mm; phyllaries 8, glabrous, greenish, 6–7 × ca. 1 mm. Ray florets 3 to 5, corolla ligulate, glabrous, tube ca. 5 mm, ligule 5–7 × 2–3 mm. Disk florets 5 to 10, corolla funnelform, glabrous, 7–8 mm, tube 3–4 mm, throat ca. 2 mm, lobes ca. 2 mm. Achenes sparsely pubescent, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 5–6 mm.

Distribution and phenology. Southern Mexico and Guatemala (Guatemala: Chimaltenango, Huehuetenango, Quezaltenango, Sacatepéquez, San Marcos, Sololá, Suchitepéquez, Totonicapán; Mexico: Chiapas) at elevations of 1400–3300(3800) m. Montane cloud forests. Flowering December through March.

Comments. *Roldana acutangula* (Hemsl.) H. Rob. & Brettell is a nomen illegitimum under the *International Code of Botanical Nomenclature* (Greuter et al., 2000: Art. 33.6, Ex. 11). In attempting to make their combination, Robinson and Brettell (1974) did not correctly reference the basionym author, and as Hemsley (1881) provided an explicit reference to Bertolini (1840), Art. 33.6(a) (Greuter et al., 2000) does not apply and the combination under *Roldana* was not validly published by H. Robinson and Brettell. In the protologue for *Cineraria acutangula*, Bertolini (1840) listed the specimen "in Vulcano d'acqua. Perenn." Annalisa Managlia at BOLO (pers. comm., June 2003) confirms the existence of the *J. Velasquez s.n.* specimen and further notes that some fragments of it are housed at US.

The angulate stems of *Roldana acutangula* are unique. The only other species in which this occurs, on occasion, is *R. hartwegii*. Morphologically, *R. acutangula* is most similar to *R. jurgensenii* (Hemsl.) H. Rob. & Brettell. They differ in types of pubescence, stem shape, leaf base, phyllary pubescence, and achene pubescence. *Roldana acutangula* has an arachnoid floccose pubescence of long thin white hairs, whereas *R. jurgensenii* has short-stipitate and long multicelled brown hairs that vary from sparse to dense. For the remaining diagnostic characters, *R. acutangula* has angulate stems, leaf base cordate, phyllary pubescence glabrous, and pubescent achenes; *R. jurgensenii* has terete stems, leaf base truncate or weakly cordate, phyllary pubescence stipitate-glandular, and glabrous achenes.

Representative specimens examined. GUATEMALA. **Chimaltenango:** mpio. Chimaltenango, Chichavae, 1500 m, 5 Dec. 1933, *A. F. Skutch 739* (MO). **Huehuetenango:** Sierra de los Cuchumatanes, rd. beyond La Pradera, Km 32, 3300 m, 31 Dec. 1940, *P. C. Standley 81758* (F); 2.5 mi. E of San Mateo Ixtatán, Sierra de los Cuchumatanes, 2500 m, 31 July 1942, *J. A. Steyermark 49886* (F, MO). **Quezaltenango:** Km 172 on hwy. to entrance Quezaltenango, Huehuetenango & Totonicapán, 2860 m, 10 Jan. 1974, *Molina R. 30209* (F, MICH); betw. Fuentes Georginas & Zunil, 2500 m, 4 Mar. 1939, *P. C. Standley 67334* (MO). **Sacatepéquez:** Volcán Acatenango, 2500 m, 15 Feb. 1937, *Hunnewell 14922* (GH). **San Marcos:** mtns. along the rd. betw. San Marcos & Serchill, 2900 m, 30 Jan. 1941, *P. C. Standley 85378* (F). **Sololá:** betw. María Tecun & Los Encuentros, 2800 m, 29 Nov. 1969, *Molina R. 24990* (F). **Suchitepéquez:** SW lower slopes of Volcán Zunil, vic. of Finca Asturias, NE of Pueblo Nuevo, 1250 m, 1 Feb. 1940, *J. A. Steyermark 35335* (F). **Totonicapán:** hwy. CA1 betw. Huehuetenango & Chimaltenango, betw. jct. in rd. to Quezaltenango & Nahuala ca. 10 mi. SSE of jct. to Quezaltenango, 14°52′N, 91°24′W, 2950 m, 23 Jan. 1987, *T. B. Croat 63497* (KSC); entre Los Encuentros y María Tecun, 3800 m, 21 Nov. 1965, *Molina R. 15881* (F, MO, NY). MEXICO. **Chiapas:** in the paraje of Las Ollas, mpio. Chamula, 2600 m, 20 Dec. 1964, *D. E. Breedlove 8012* (DS, MICH, MO, NY).

2. **Roldana albonervia** (Greenm.) H. Rob. & Brettell, Phytologia 27: 415. 1974. *Senecio albonervius* Greenm., Ann. Missouri Bot. Gard. 1: 275. 1914. TYPE: Mexico. México: Valley of Temascáltepec, Apr. 1831, *C. J. W. Schiede s.n.* (lectotype, designated here, GH!).

Shrubs to small trees, 3–7 m tall; old growth woody, brown, terete, sparsely lenticeled; new growth purplish red, terete, arachnoid floccose to tomentose, glabrate with age; the contrast between old and new growth may give the stem a foreshortened appearance. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 5–10 cm, glabrate to arachnoid floccose; blades 10–17 × 8–14 cm, blades triple-nerved, broadly cordate to ovate, 5- to 9-lobed, sinus depth less than 1/4 of the way to the midrib, lobes acute, margins glabrous; pubescent on both surfaces, mature leaves glabrate with hirsute hairs on veins, may be tomentose on abaxial surface. *Capitulescence* pyramidal compound paniculate cyme, 20 to 60 capitula per branch, peduncles arachnoid floccose to glabrate, primary bracts linear, 2–5 mm, ultimate peduncles 2–6 mm, bracteoles ca. 2, linear, 1–2 mm. *Capitula* radiate, 10–15 × ca. 3 mm, turbinate, corollas bright yellow; calyculate bracts 3 to 5, linear, 1–2 mm; phyllaries 8, glabrate, 5–8 × ca. 1 mm. Ray florets 3 to 5, corolla ligulate, glabrous, tube 4–5 mm, ligule 5–6 × up to 2.5 mm. Disk florets 7 to 12, corolla narrow, glabrous (sparsely pubescent at tip of corolla lobes), 8–11 mm, tube 3–5 mm, throat 3–4 mm, lobes ca. 1 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 5 or 10, resin glands absent; pappus bristles 6–8 mm.

Distribution and phenology. Central Mexico (Hidalgo, Jalisco, México, Michoacán, Morelos, Puebla, Veracruz) at elevations of 1500–3200(4100) m. Found in pine-oak-fir forests, open woods, and margins of open grassland. Flowering February through April.

Comments. Greenman (1914) cites B and GH as the type material for *Roldana albonervia*; the Berlin specimen was destroyed in 1943.

Roldana albonervia is a common species along the roadsides of central Mexico. It is very similar to *R. aschenborniana*; however, there are several characters that can be diagnostic: while both are shrubs, *R. albonervia* has the propensity to become small trees reaching 7 m tall; the peduncles of *R. albonervia* have a floccose pubescence versus a persistent pubescence in *R. aschenborniana*; and the phyllary number in *R. albonervia* is 8 versus 13 in *R. aschenborniana*.

Representative specimens examined. MEXICO. **Hidalgo:** 23 km N of rd. Pachuca Zacualtipán, mpio. Mineral del Chico, 2630 m, 24 Mar. 1978, *García P. 613* (CAS, F, MICH, UC). **Jalisco:** plateau center 4–5 km NNE of Cerro San Miguel & 4 km S of Rincón de Manantlán, NE of crossroads on plateau, Sierra de Manantlán Central, 19°33–35′N, 104°10–12′W, 2200 m, 13 Jan. 1980, *R. R. Kowal 2844* (MICH, WIS). **México:** NW slope of Mt. Popocatépetl, ca. 13 km E of Amecameca on rd. to Tlamacas, 2800 m, 23 Mar. 1984, *T. M. Barkley 3002* (KSC, NY); Ixtaccíhuatl, 2430–3350 m, Mar.–July 1903, *C. A. Purpus 201* (GH, MO). **Michoacán:** W of Mil Cumbre, ca. 16 km E of San Jose de la cumbre on rd. to Morelia, 10°37′47″N, 100°47′44″, 2270 m, 25 Mar. 1996, *T. M. Barkley 4033* (KSC, MO). **Morelos:** Sierra de Tres Marías, 3050 m, 15 Apr. 1904, *C. G. Pringle 8903* (F, GH, MICH, MO). **Puebla:** ca. 38 km W of Huejotzingo on Fed. hwy. 190 Libre, ca. 4 km E of Puebla–Mexico state line, 2800 m, 22 Mar. 1984, *T. M. Barkley 2998* (NY). **Veracruz:** Mineral del Monte, *C. Ehrenberg 324* (fragm. at GH).

3. Roldana aliena (B. L. Rob. & Seaton) Funston, comb. nov. Basionym: *Senecio alienus* B. L. Rob. & Seaton, Proc. Amer. Acad. Arts 28: 110. 1893. TYPE: Mexico. Michoacán: Mtns. near Pátzcuaro, 22 Dec. 1891, *C. G. Pringle 5056* (holotype, GH!).

Senecio chrismarii Greenm., Ann. Missouri Bot. Gard. 1: 278. 1914. Syn. nov. TYPE: Mexico. [s. loc.], *Chrismar s.n.* (lectotype, designated here, B fragm. & tracing GH!, B photos F!, GH!, MICH!, MO!, NY!, B tracing MO!).

Simple or sparingly branched suffruticose herbs, 0.5–2 m tall; plants sparsely to densely pubescent with stipitate-glandular and multicelled hairs; stems terete, striate, red or brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 5–11.5 cm, glabrate to sparsely pubescent; blades 6–10.5 × 8.5–11 cm, eccentrically peltate or marginally attached to petiole, triplenerved, hastate to subhastate, 3 primary lobes, weakly secondarily lobed to denticulate, margins sparsely pubescent; abaxially glabrate with glandular hairs on veins, adaxially glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* simple to compound paniculate cyme, 3 to 10 capitula per branch, peduncles densely stipitate-glandular pubescent and sparsely so with long multicelled hairs, primary bracts linear, 5–10 mm, ultimate peduncles 10–30 mm, bracteoles 1 to 3, linear or filiform, 1–3 mm. *Capitula* radiate or eradiate, 10–15 × 2–5 mm, funnelform, corollas yellow; calyculate bracts filiform or linear, 1–2 mm; phyllaries 8, stipitate-glandular pubescent, ca. 10 × 2 mm. Ray florets absent or 3 to 5, ligule inconspicuous, glabrous, tube ca. 2 mm, ligule ca. 3 × 0.5 mm. Disk florets 14 to 23, corolla funnelform, glabrous, ca. 9 mm, tube ca. 4 mm, throat ca. 4 mm, lobes ca. 1 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 5 or 10, resin glands absent; pappus bristles ca. 8 mm.

Distribution and phenology. Central Mexico (México, Michoacán) at elevations of 2000–2500 m. Found in pine-oak forests. Flowering December through February.

Comments. In the protologue for *Senecio chrismarii*, Greenman (1914) listed the type material as being a specimen in B and a tracing and fragments in GH. Comments made by Greenman (1914: 279), in the protologue of *S. kerberi*, make it known that he took fragments and tracings of the Berlin specimens; therefore, the GH tracing and fragments are deemed an isotype at the time of publication.

Roldana aliena is part of a complex of plants that has been difficult to circumscribe due to the small number of collections available. The complex includes *R. aliena*, *R. anisophylla*, and *R. hederifolia*. The relevant characters used to distinguish these species are marginal versus eccentrically peltate blade attachment, the presence or absence of ray florets, and the distribution of each species.

Roldana aliena was described by B. L. Robinson and Seaton (1893) as having eccentrically peltate, triangular-ovate leaves with 3 to 5 angulate lobes, eradiate capitula, and with the type collection from Michoacán. I have extended this description to include radiate capitula with inconspicuously ligulate rays based on three collections from México that I believe belong to the species concept of *R. aliena*.

The lectotype designation for *Senecio chrismarii* was made according to the *International Code of Botanical Nomenclature* (Greuter et al., 2000: Art. 8.3, Ex. 5). The type collection is from an unknown location. *Senecio chrismarii* was described by Greenman (1914) as having leaf blades marginally attached to the petiole and triangular-ovate with 3 to 5 hastate lobes, and eradiate capitula. Greenman separates this species from *Roldana aliena* based on its having deeply cordate leaves and the absence of peltation. In my concept of the genus, this form is not enough to distinguish the species, so it is placed in synonymy.

Representative specimens examined. MEXICO. **México:** Avendero, Valle de Bravo, 2000 m, 8 Jan. 1955, *Carlson*

2895 (F); Nanchititla, Temascáltepec, 23 Feb. 1933, *G. B. Hinton 3137* (F, GH, MO, NY); Zacualpan, 2100 m, 31 Jan. 1954, *Matuda 30217* (NY). **Michoacán:** 35 rd. mi. E of where aqueduct ends in Morelia, 2500 m, 14 Feb. 1965, *E. S. Gibson 1022* (F, KSC, MICH); 36.5 mi. E of Morelia, 2500 m, 20 Feb. 1965, *E. S. Gibson 1033* (KSC, MICH).

4. **Roldana angulifolia** (DC.) H. Rob. & Brettell, Phytologia 27: 415. 1974. *Senecio angulifolius* DC., Prodr. 6: 431. 1837 [1838]. TYPE: Mexico. México: ca. Toluca, flor. Apr., *G. Andrieux 297* (lectotype, designated by McVaugh, 1984: 809, G-DC not seen, microfiche IDC 800. 1142 II 6!, G-DC photos F!, MICH!, MO!).

Cacalia berlandieri DC., Prodr. 6: 328. 1837 [1838]. *Senecio berlandieri* (DC.) Sch. Bip., Flora 28: 498. 1845, non *Senecio berlandieri* (DC.) Hemsl., 1881, nom. illeg. *Senecio desertorum* Hemsl., Biol. Cent.-Amer., Bot. 2: 239. 1881, nom. superfl. TYPE: Mexico. Querétaro: "in Mexicanae vallis montanis ad Viejo desierto," *J. L. Berlandier 1166* (holotype, G-DC not seen, microfiche IDC 800. 1100 II 15!, G-DC photos F!, MICH!).

Senecio acerifolius K. Koch, Wochenschr. Vereines Beford. Gartenbaues Konigl. Preuss. Staaten 4: 237. 1861. TYPE: same as that of *Senecio angulifolius* DC. (neotype, designated here, G-DC not seen).

Senecio adenolepis Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 281. 1907. Syn. nov. TYPE: Mexico. Morelos: Sierra de Tepoztlán, 7500 ft., 13 Feb. 1907, *C. G. Pringle 13909* (holotype, GH!).

Senecio angulifolius var. *ingens* Greenm., Ann. Missouri Bot. Gard. 1: 276. 1914. TYPE: Mexico. México: Mt. Ixtaccíhuatl, above timber line, Mar.–July 1903, *C. A. Purpus 193* (holotype, MO!; isotype, US not seen).

Sparingly branched shrublets to tree-like shrubs, 2–7 m tall, typically found growing as solitary stems connected by root stocks; new growth variously pubescent with stipitate-glandular and long multicelled hairs; stems terete, maculate, reddish brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 4–15 cm, typically densely pubescent; blades 12–18 × 16–25 cm, marginally attached to petiole or sometimes eccentrically peltate, triple-nerved, palmatifid, 5- to 7(13)-lobed, sinus depth 1/2 to less than 1/2 of the way to the midrib, lobes acute, secondarily lobed; surfaces densely pubescent to glabrate with hairs persisting on veins and blade margin. *Capitulescence* a loose compound paniculate cyme, 10 to 20 capitula per branch, peduncles densely stipitate-glandular pubescent, primary bracts typically sessile, variously elliptic, 15–30 × ca. 8 mm, ultimate peduncles 10–30 mm, bracteoles ca. 1, variously ovate, ca. 10 × 2–4 mm. *Capitula* radiate or eradiate, 10–15 × 4–6 mm, campanulate, corollas yellow; calyculate bracts ca. 3, variously oblong, 5–15 × ca. 2 mm, may have ciliate margins of long multicelled hairs; phyllaries 8 to 11, densely stipitate-glandular pubescent, 9–13 × 1.3–2 mm. Ray florets absent or 5 to 7, corolla inconspicuously ligulate or reduced to a tube, glabrous, tube 4–5 mm, ligule 3–5 × 1–2 mm; when reduced, tube 2–3 mm. Disk florets 12 to 22(40), corolla narrow, glabrous, ca. 10 mm, tube ca. 4 mm, throat ca. 5 mm, lobes ca. 1.5 mm. Achenes glabrous, cylindrical, ca. 3 mm, ribs 10, resin glands absent; pappus bristles 8–9 mm.

Distribution and phenology. Mexico (Distrito Federal, Durango, Guerrero, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz) at elevations of 1200–3400 m. Found in pine-oak or fir forests and in mixed shrub-grassland at higher elevations. Flowering February through April.

Comments. Regarding the lectotypification of *Roldana angulifolia*, de Candolle's protologue lists the specimens *E. M. Mairet s.n.* and *G. Andrieux 297*, but he did not designate a holotype.

The type of *Senecio acerifolius* K. Koch was destroyed in the Berlin herbarium fire of 1943: Mexico. [s. loc. & s. coll.], *Herb. Hort. Reg. Berolinensis 6153* (B tracings GH!, MO!). Greenman notes on the GH tracing, in hand, "appears to be of type material from Berlin." Because no specimen of the type material is believed extant, the name is neotypified here, making it homotypic with *Roldana angulifolia*. The epithet can be confused with its multiple heterotypic homonyms, which include *S. acerifolius* Hemsl., 1881, *S. acerifolius* C. Winkl., 1893, and *S. acerifolius* Klatt, 1894.

Under *Cacalia berlandieri*, *Senecio berlandieri* (DC.) Hemsl., Biol. Cent.-Amer., Bot. 2: 236. 1881 is a heterotypic nom. illeg. based on *Gynoxis berlandieri* DC., 1837. Hemsley made the superfluous nomen novum *S. desertorum* believing that he had blocked the transfer of *C. berlandieri* to *Senecio*, when in actuality the combination had already been made in 1845.

Roldana angulifolia is most similar to *R. cordovensis* but is distinct in that it has obovate calyculate bracts and leaf blades typically marginally attached to petiole. *Roldana cordovensis* has linear to filiform calyculate bracts and eccentrically peltate leaf blade attachment. The obovate calyculate and primary bracts exhibited by *R. angulifolia* are unique diagnostic features. The combination of these two bract types is found in only one other species, *R. grimesii*, from which it is easily distinguished.

Roldana angulifolia is one of the more beautiful and widespread species in the genus; therefore, it is not surprising that there are several synonyms associated with it. In his original description, de Candolle (1837) listed *Cineraria angulata* Mairet ex DC., nom. nud., in synonymy. Greenman created the

variety *Senecio angulifolius* var. *ingens* based on its having fewer and larger capitula than the typical variety, stating that the capitula of the variety is 1.5–2 cm high and 40- to 50-flowered. While there are specimens with the above characters, there is no correlation of these characters with distribution. The possibility of this larger growth being due to an excellent growing environment cannot be excluded. Based on this possibility and an examination of the holotype, the variety is placed in synonymy. *Cacalia berlandieri* was placed in synonymy by McVaugh (1984) and Gibson (1969); upon examining photographs of the holotype, it is also placed in synonymy here. *Senecio acerifolius* K. Koch was placed in synonymy by Greenman (1926); upon examining the type material for this species it is again relegated to synonymy. *Senecio adenolepis* was placed in synonymy under *S. chapalensis* by Gibson (1969). My examination of the holotype places the entity within *R. angulifolia*. It has obovate bracts and leaf blades marginally attached to petiole. These characters are not diagnostic of *S. chapalensis*; therefore, it is placed in synonymy here.

Representative specimens examined. MEXICO. **Distrito Federal:** on hwy. 15, W of Mexico City, just W of La Venta, 27 Dec. 1970, *Dunn 17068* (MO). **Durango:** Puerto Buenos Aires, 53 km W of El Salto, along hwy. Durango–Mazatlán, 2650 m, *J. García P. 902* (MO). **Guerrero:** Cerro Teotepec, mpio. Tlacotepec, about 40 km N of Coyuca de Benitez, 3350 m, 5 Dec. 1963, *Feddema 2922* (MICH, NY). **Guanajuato:** ca. 3 km E of Santa Rosa, 2650 m, 11 Nov. 1970, *R. McVaugh 24200* (MICH). **Hidalgo:** 6 km N de Pachuca, 3000 m, 31 Dec. 1963, *J. Rzedowski 18227* (DS, MICH); hills of Santa Ana, mpio. Tepeapulco, 2900 m, 17 Dec. 1975, *Ventura A. 727* (CAS, MO). **Jalisco:** 25–30 km SE of Autlan, on lumber rds. E of rd.-cross called "La Cumbre," betw. El Chante & Cuzalapa, 19°35′N, 104°8–15′W, 2600 m, 20 Mar. 1965, *R. McVaugh 23136* (MICH). **México:** 3.1 mi. W of summit of divide on toll rd. to Puebla, 3 Jan. 1972, *Dunn 18779* (MO). **Michoacán:** on Michoacán site of Mex–Mich border, Km 71 W of Toluca, 19°26′26″N, 100°11′38″W, 2585 m, 24 Mar. 1996, *T. M. Barkley 4034* (KSC, MO). **Morelos:** mtns. Tres Marias, 3000 m, 20 Feb. 1932, *Froderstrom 253* (F). **Nuevo León:** mpio. Zaragoza, ca. 1.5 mi. NE of Puerot Pinos on NE side of Cerro Pena Nevada, rd. from San Antonio Pena Nevada to Zaragoza, 2550 m, 24°48′N, 99°51′W, 25 Aug. 1989, *G. Nesom 7125* (MO). **Oaxaca:** N slope of Sierra San Felipe, ca. 30 km NE of Tlacolula, 2900 m, 12 Nov. 1970, *A. Cronquist 10907* (NY). **Puebla:** Mt. Popocatepetl, W-facing slope, 4100 m, 9 Feb. 1965, *E. S. Gibson 995a* (F, KSC, MICH). **Querétaro:** mpio. of Calon, 1 km SW al la cumbre, 3100 m, 13 Nov. 1971, *J. Rzedowski 407* (CAS, MICH). **San Luis Potosi:** E front Sierra Madre Oriental, ca. 15 km NW of Cardenas, on rd. to Ciudad del Maiz, 1200 m, 26 Oct. 1980, *A. Cronquist 11748* (CAS, GH, KSC, MICH, MO). **Tlaxcala:** above Hacienda de San Diego de Pinar, Mt. Malinche, 3000 m, 12 Oct. 1938, *Balls 5633* (F, GH, MICH). **Veracruz:** 1–2 km above Escobillo on the NW slopes of Cofre de Perote, mpio. Perote, 19°31′N, 97°12′W, 3300 m, 21 Jan. 1984, *Taylor 166* (KSC, NY, WIS).

5. Roldana anisophylla (Klatt) Funston, Novon 11: 305. 2001. *Senecio anisophyllus* Klatt, Leopoldina 24: 124. 1888. TYPE: Mexico. Oaxaca: Pelado, *F. M. Liebmann 160* (holotype, C not seen, C drawing GH!, C tracing MO!).

Roldana cronquistii H. Rob. & Brettell, Phytologia 27: 417. 1974. *Senecio cronquistii* (H. Rob. & Brettell) B. L. Turner & T. M. Barkley, Phytologia 67: 392. 1989. TYPE: Mexico. Oaxaca: in wet forest 1 mi. or less S of pass betw. Oaxaca & Tuxtepec, 65 mi. N of Oaxaca, 9300 ft., 11 Oct. 1962, *A. Cronquist 9648* (holotype, US not seen, US photos F!, GH!, MO!; isotypes, GH!, KSC!, MICH!, NY!).

Simple to sparingly branched suffruticose herbs or subshrubs, 0.5–2.0 m tall; new growth sparsely to densely pubescent with stipitate-glandular and multicelled hairs, terete, striate, red or brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 4–8(–15) cm, glabrate to sparsely pubescent; blades 10–18 × 5–10(–15) cm, triple-nerved, hastate, margins weakly secondarily lobed to denticulate, sparsely pubescent; abaxially glabrate with glandular hairs on veins, adaxially glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* simple paniculate cyme, ca. 10 capitula per branch, peduncles densely stipitate-glandular with sparse pubescense of long multicelled hairs, primary bracts linear, 5–15 mm, ultimate peduncles 10–25 mm, bracteoles ca. 2, linear, 2–5 mm. *Capitula* radiate, 10–15 × ca. 4 mm, turbinate, corollas yellow; calyculate bracts 0 to 3, linear, 2–5 mm; phyllaries 8, stipitate-glandular, 8–10 × ca. 2 mm. Ray florets 4 to 5, corolla ligulate, glabrous, tube 5–7 mm, ligule 10–13 × 2–4 mm. Disk florets 20 to 23, corolla weakly campanulate, glabrous, ca. 10 mm, tube ca. 4 mm, throat ca. 4 mm, lobes ca. 2 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 5 or 10, resin glands absent; pappus bristles ca. 9 mm.

Distribution and phenology. Southern Mexico (Oaxaca) at elevations of 2800–3000 m. Found in pine-oak forests. Flowering August through December.

Comments. These are beautiful plants with sharply hastate, deep green leaves and large, bright yellow rays. Robinson and Brettell (1974) described the species *Roldana cronquistii*, in part, based on their agreement with Gibson's (1969) circumscription of *R. hederifolia* (Hemsl.) H. Rob. & Brettell where he reduced *R. aliena*, *Senecio chrismarii*, and *R. anisophylla* to synonymy. However, *R. anisophylla* is a distinct species, and, on examining the protologue and type material of all species, *R. cronquistii* is placed in synonymy under *R. anisophylla*.

Representative specimens examined. MEXICO. **Oaxaca:** 43 km N of Ixtlan–de Juarez jct., on rd. to Valle Nacional,

2870 m, 11 Sep. 1983, *D. E. Breedlove 60015* (CAS); carretera Ciudad de Oaxaca a Tuxtepec, cerca del Cerro Pelon, mpio. Yolox, 2850 m, 18 Jan. 1989, *Cházaro B. 5809* (WIS); mpio. Comaltepec, rd. to Cerro Pelón, 4.5 km NE of Santiago Comaltepec, 17 35′10″N, 96 30′45″W, 2820 m, 28 Mar. 1996, *C. Gallardo h. 1781* (MO); Km 130 Oaxaca–Tuxtepec en la carretera, 10 Sep. 1963, *MacDougall s.n.* (CAS); mpio. San Felipe Usila, parteaguas S cuenca del Río Perfume, 10.8 km S Santa Cruz Tepetotutla, 17 38′48″N, 96 31′26″W, 2650 m, 20 Dec. 1993, *A. Rincón G. 206* (MO).

6. Roldana aschenborniana (S. Schauer) H. Rob. & Brettell, Phytologia 27: 415. 1974. *Senecio aschenbornianus* S. Schauer, Linnaea 20: 698. 1847. TYPE: Mexico. [state unknown]: in Valle Toluca, *Aschenborn [718]* (lectotype, designated here, B fragm. & tracing GH!).

Senecio quezalticus L. O. Williams, Phytologia 31: 446. 1975. Syn. nov. *Roldana quezaltica* (L. O. Williams) H. Rob., Phytologia 32: 331. 1975. TYPE: Guatemala. Quezaltenango: mtns. SE of Palestina, on old rd. to San Juan Ostuncalco, 2550–2850 m, 21 Jan. 1941, *P. C. Standley 84286* (holotype, F!; isotype, MO!).

Roldana mazatecana B. L. Turner, Phytologia 87: 235. 2005 [2006]. Syn. nov. TYPE: Mexico. Oaxaca: Sierra Mazateca, "Aprox. 400 m del Puerto de la Soledad, por la carretera de Huautla a Teotitlan de Flores magon (Mex 182)," ca. 2320 m, 13 Feb. 2002, *Mun-Estrada & Mendoza 1947* (holotype, TEX not seen, TEX digital image TEX!; isotype, MEXU not seen).

Shrublets or shrubs, 1–3 m tall; new growth reddish variously tomentose to glabrate with woolly hairs; stems terete, bark brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 2–5 cm, densely pubescent; blades 5–12 × 3.5–10 cm, triple-nerved, ovate, oval, or rotund, mostly 5- to 9(to 13)-lobed, sinus depth less than 1/4 of the way to the midrib to subentire, margins weakly serrate to denticulate; abaxially pubescent to lanate tomentose and canescent, adaxially glabrate, arachnoid hairs on veins. *Capitulescence* pyramidal compound paniculate cyme, 20 to 50 capitula per branch, peduncles lanate tomentose to sparsely floccose tomentose, primary bracts linear, 2–5 mm, ultimate peduncles 5–10 mm, bracteoles 0 to 3, linear to scalelike, 1–2 mm. *Capitula* radiate, 6–10 × 2–3 mm, turbinate, corollas yellow; calyculate bracts 3 to 5, linear, 1–2 mm; phyllaries ca. 13, glabrous, 4–6(–7) × ca. 1 mm. Ray florets 6 to 8, corolla ligulate, glabrous, tube 3–5 mm, ligule 4–6 × ca. 2 mm. Disk florets 12 to 16, corolla narrow, glabrous, 5–8 mm, tube 2–5 mm, throat 1.5–2 mm, lobes 0.5–1 mm. Achenes glabrous, cylindrical, ca. 1 mm, ribs 10, resin glands absent; pappus bristles 4–7 mm.

Distribution and phenology. Eastern Mexico and Guatemala (Guatemala: Chimaltenango, Huehuetenango, Quezaltenango, Sacatepéquez; Mexico: Hidalgo, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tamaulipas, Veracruz) at elevations of 1000–3200 m. Found on gulf slopes in pine-oak and fir forests. Flowering November through April.

Comments. Schauer (1847) lists *Aschenborn s.n.* from "valle Toluccana" in the protologue for *Senecio aschenbornianus*, and there is an *Aschenborn 718* specimen at GH that is a fragment and drawing of the presumed destroyed B type material. Gibson (1969: 54) notes that J. M. Greenman on *Liebmann 153* (GH) states "compares well with the type number Aschenborn's no. 718 at herb. Mus. Bot. Berlin." Gibson further remarks that a drawing of *Liebmann 153* (GH-Klatt) has a notation, perhaps by the artist, that *Aschenborn 718* is the type. There is a possible type specimen at Kew; in Hemsley's treatment of *Senecio*, he (1881: 236) reported seeing *Aschenborn s.n.* (K) from Toluca. However, the specimen was not readily found by Nicholas Hind (pers. comm., April 2007).

Roldana aschenborniana most resembles *R. hirsuticaulis* and *R. albonervia*. For further elaboration, see the discussion sections under those species.

Williams (1975) described *Senecio quezalticus* stating that it is closely related to *Roldana barba-johannis* but differs in leaf shape and pubescence from that species. While the description of *S. quezalticus* certainly separates it from *R. barba-johannis*, it does not separate it from *R. aschenborniana*. He also notes that the two specimens he would put into *S. quezalticus* were annotated by Gibson as belonging to *R. aschenborniana*. Upon examining the holotype and paratypes of *S. quezalticus*, it is placed in synonymy.

Roldana mazatecana is placed in synonymy here as there is nothing to distinguish it from my concept of *R. aschenborniana*. However, it may represent a variety that correlates rounded leaf blades with ca. 13 shallow, acute lobes and a southern distribution versus a more ovate leaf blade with 5 to 9 acute lobes and a northern distribution, for the typical variety.

Representative specimens examined. GUATEMALA. **Chimaltenango:** Cerro de Tecpán, region of Santa Elena, 2550 m, 26 Dec. 1938, *P. C. Standley 61093* (F, MO); Chichavac, 2600 m, 6 Mar. 1933, *A. F. Skutch 319* (A, MICH). **Huehuetango:** rd. to San Pedro Soloma, 3 mi. SW of San Mateo Ixtatán, mpio. of San Mateo Ixtatán, 3200 m, 6 Feb. 1965, *D. E. Breedlove 8649* (F). **Quezaltenango:** slopes of Volcán de Santa María, above Palojunoj, 2400–3768 m, 6 Mar. 1938, *P. C. Standley 67593* (F). **Sacatepéquez:** slopes of Volcán de Agua, above Santa María de Jesús, 2250–3000 m, 11 Feb. 1939, *P. C. Standley 65185* (F); lower N slopes of Volcán de Santa María, above Palojunoj, 2600 m, 15 Jan. 1941, *P. C. Standley 83436* (F, MO). MEXICO. **Hidalgo:** hwy. 85, Km 236 San Jacala, 2300 m, 30 Dec. 1954, *Carlson 2796* (F); 3 km al W de Zacualtipán, 2000 m, 16 Jan. 1971, *Diaz M. 270* (DS, MICH, MO). **Nuevo León:** Sierra Madre Oriental, Puerto Blanco to Taray, ca. 15 mi.

SW of Galeana, 23 July 1934, *Mueller 1211* (A, F, MICH, NY). **Oaxaca:** rd. from Teotitlan del Camino to Huatla de Jimenez, 17.8–18.3 mi. E of Teotitlan, 2300 m, 22 Feb. 1979, *T. B. Croat 48210* (MO). **Puebla:** 6 km al NE de Zacapoaxtla, sobre la carretera a Cuetzalan, 1500 m, 2 Feb. 1975, *J. Rzedowski 32760* (ASU). **Querétaro:** barranca de Amealco, 2350 m, 21 Feb. 1977, *Arguelles 730* (CAS, KSC). **San Luis Potosí:** Mex. Hwy. 80, 16.2 mi. E of El Naranjo, 1.5 mi. E of El Platanito, 17 km (by air) NE of Ciudad del Maiz toward Antiguo, Morelos, 22°30′N, 99°28′W, 980 m, 22 Mar. 1976, *Hansen 3809* (MICH, WIS); chiefly in the region of San Luis Potosí, 2500 m, 20 Feb. 1905, *Palmer 539* (F, GH, MO, NY). **Tamaulipas:** on Jaumave rd. ca. 13 mi. SW of Ciudad Victoria, near summits of Sierra Madre, 1000 m, 9 Mar. 1949, *R. McVaugh 9859* (MICH, NY); Dulces Nombres, Nuevo León, & just E of border into Tamaulipas, 1690 m, 18 July 1948, *Meyer 2800* (GH, MO). **Veracruz:** Choubla, Mar. 1910, *Daidinas 521* (MO); hills above & NE of Xico Viejo, 8 km NW of Xico, mpio. Xico, 19°28′N, 97°04′W, 1800 m, 5 Feb. 1984, *M. Nee 29360* (KSC, NY).

7. **Roldana barba-johannis** (DC.) H. Rob. & Brettell, Phytologia 27: 415. 1974. *Senecio barba-johannis* DC., Prodr. 6: 430. 1837 [1838]. TYPE: Mexico. México: around Toluca, *E. M. Mairet & G. Andrieux 290* (lectotype, designated by McVaugh, 1984: 812, G-DC not seen, microfiche IDC 800. 1142 I 1!, G-DC photos F!, MICH!, MO!).

Senecio grahamii Benth., Pl. Hartw. 18. 1839, as "grahami." TYPE: Mexico. [state unknown]: "mexican highlands." *Graham s.n.* (holotype, K not seen; isotype, GH!).

Senecio pullus Klatt, Abh. Naturf. Ges. Halle 15: 333. 1882. non *Senecio pullus* Klatt, 1896, nom. illeg. TYPE: Mexico. [state unknown]: Cordillera Guchiloque, *J. L. Berlandier 1177* (holotype, G-DC 534c not seen; isotype, G-DC fragm. & drawing GH!).

Senecio donnell-smithii J. M. Coult., Bot. Gaz. 16: 101. 1891. Syn. nov. *Roldana donnell-smithii* (J. M. Coult.) H. Rob. & Brettell, Phytologia 27: 418. 1974. TYPE: Guatemala. Sacatepéquez: Volcán de Agua, 11,000 ft., Apr. 1890, *John Donnell Smith 2362* (holotype, K not seen; isotypes, F!, MO!).

Branching woody shrubs or small trees, 1–6 m tall, new growth variously lanate tomentose; stems arising from a woody caudex, terete, reddish brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 4–8(–15) cm, tomentose to densely pubescent; blades 10–18 × 5–10(–15) cm, pinnate to pinnipalmate, variously lanceolate, ovate, or oval, margins subentire, repand-sinuate or occasionally weakly 5- to 9-lobed, variously denticulate; abaxially densely lanate tomentose and canescent, rarely weakly tomentose, adaxially glabrous to pubescent along veins. *Capitulescence* pyramidal compound paniculate cyme, 20 to 40 capitula per branch, peduncles persistently tomentose, primary bracts linear, 3–5 mm, ultimate peduncles 2–10 mm, bracteoles 0 to 2, linear, 1–2 mm. *Capitula* radiate or eradiate, 7–10 × ca. 3 mm, campanulate, corollas yellow; calyculate bracts ca. 5, linear, 1–3 mm; phyllaries 10 to 13, tomentose, arachnoid to glabrate with age, ± purple coloration on phyllary tips, 4–5 × ca. 1 mm. Ray florets absent or 1 to 8, corolla ligule prominent or inconspicuous, glabrous, tube 2–3 mm, ligule 4–7 × ca. 2 mm, or when inconspicuous florets 1 to 3, ligule ca. 2 × 0.5 mm. Disk florets (10 to)13 to 18(to 21), corolla funnelform to moderately campanulate, glabrous, 6–9 mm, tube 2.5–4 mm, throat 2–4 mm, lobes 0.5–2 mm. Achenes glabrous, cylindrical, 1–2 mm, ribs 5, resin glands present; pappus bristles 6–9 mm.

Distribution and phenology. Central Mexico extending southward into Guatemala along the Sierra Cuchumatanes and Honduras (Guatemala: Chimaltenango, Huehuetenango, El Progreso, Quezaltenango, Sacatepéquez, Sololá; Honduras: Lempira; Mexico: Chiapas, Distrito Federal, Durango, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Oaxaca, Puebla, Sinaloa, Tlaxcala, Veracruz) at elevations of 2400–4100 m. Found on steep humid mountainsides, barrancas, often in dense shrubby understory of secondary pine-oak forests, and in deep shade. Also found on rich, dark volcanic soils, rocky ravines, and grazed forests. Associated with *Quercus*, *Pinus*, *Alnus*, *Abies*, *Senecio*, *Solanum*, *Penstemon*, *Physalis*, *Potentilla*, *Eryngium*, *Liquidambar*, *Cupressus*, and *Chiranthodendron*. Flowering December through April.

Comments. In the protologue for *Senecio barba-johannis*, de Candolle (1837) made the notation "Mairet!" I agree with McVaugh's (1984) selection of the *E. M. Mairet & G. Andrieux 290* (G-DC) specimen for the lectotype.

Roldana barba-johannis is closely associated with *R. lanicaulis*, *R. robinsoniana*, and *R. lobata* due to its overall pubescence and general habit. When present, the lanceolate leaf shape is distinct among these four species. In his treatment of *Senecio* for Standley, Greenman remarks (1926: 1627) that "the sap of the thick stems is sometimes resorted to by travelers as a substitute for drinking water."

Senecio grahamii and *S. pullus* were placed in synonymy by Greenman (1926); after examining the type material, they are placed in synonymy here as well. Coulter recognized the species *S. donnell-smithii* based on those plants from Chiapas and Guatemala having a tendency to have involucral bracts that are glabrescent and purple-tipped. As Gibson (1969) noted, these characters are not restricted to the southern distributions. I have found that they are not consistent within the southern plants themselves. Specimens from Guatemala have been found with lanate phyllaries that are not purple-tipped. Gibson

proposed that *S. donnell-smithii* be made a variety based on the southern distribution, essentially glabrous and purple-tipped phyllaries, leaves with markedly sinuate margins, and leaves pinnately to subpalmately veined versus those that are mostly pinnately veined. Plants from the south do have a tendency to have purple-tipped phyllaries; however, their pubescence shows the same variation as those plants from the north. The leaf blade of plants from the south does have a tendency to be more sinuate and ovate, whereas leaf blades from the north have a tendency to be lanceolate and entire to subentire. The distinction between pinnately to subpalmately veined leaves and mostly pinnately veined leaves is dubious at best. Therefore, the trend within the southern plants for leaf shape and purple-tipped phyllaries is not enough to separate the plants into a species or a variety.

Representative specimens examined. GUATEMALA. **Chimaltenango:** slopes of Volcán de Acatenango, above Las Calderas, 2800 m, 3 Jan. 1939, *P. C. Standley 61862* (F, MO). **Huehuetenango:** rd. to San Pedro Soloma, 3 mi. SW of San Mateo Ixtatán, mpio. San Mateo Ixtatán, 3200 m, 6 Feb. 1965, *D. E. Breedlove 8649* (F). **El Progreso:** betw. Calera & summit of Volcán Siglo, 3000 m, 21 Jan. 1942, *J. A. Steyermark 43053* (MO). **Quezaltenango:** Canton La Esperanza about 6 km from San Juan Ostuncalco, 3200 m, 12–13 Jan. 1966, *A. Molina R. 16598* (F); region of Los Alonzo, mtns. above San Juan Ostuncalco, 2800 m, 21 Jan. 1941, *P. C. Standley 84189* (F, MO). **Sacatepéquez:** Volcán de Aqua, Apr. 1890, *Smith 2362* (F, MO). **Sololá:** Volcán Tolimán, side facing Volcán Atitlán to summit, 3000 m, 13 June 1942, *J. A. Steyermark 47558* (F, MO). HONDURAS. **Lempira:** Camino de El Mojon hasta Los Cedros, Parque Nacional de Celaque, 14°32′N, 88°42′W, 2650 m, 31 Jan. 1992, *P. House 1199* (MO). MEXICO. **Chiapas:** Barrio of Banabil, paraje of Matsab, mpio. Tenejapa, 2500 m, 6 Mar. 1965, *D. E. Breedlove 9242* (DS, F). **Distrito Federal:** ca. 2 mi. near crest of continental divide area, 25 mi. S of jct. Universidad & Churubusco, 2440 m, 28 Dec. 1970, *Dunn 17246* (MO). **Durango:** along Mexico hwy. 40, 10.6 mi. E of El Palmito, ca. 57 mi. E of Concordia, mpio. Pueblo Nuevo, 2500 m, 30 Dec. 1962, *D. E. Breedlove 4264* (DS). **Guerrero:** mpio. Minas, 2200 m, 9 May 1937, *G. B. Hinton 10170* (GH, NY). **Hidalgo:** 4.5 km E de Real del Monte, mpio. Epazoyucan, 2850 m, 29 Dec. 1964, *Gonzalez 1963* (F, WIS). **Jalisco:** top of Sierra de Manantlán plateau, along lumber rd. from N end of Sierra de Manantlán Central to Arroyo de Neverias at W end of Sierra de Manantlán Oriental, 2–4 km E of Cerro La Cumbre, 17.5 km S of El Chante, 19°33′N, 104°11–13′W, 6 Jan. 1980, *H. H. Iltis 2347* (MICH, WIS). **México:** ca. 0.5 mi. W of summit on rd. from Toluca to Temascáltepec, 2800 m, 27 Dec. 1970, *Dunn 17151* (MO); on S slope of Sierra de Ajusco, ca. 3–4 km W of Huitzilac on rd. to Zempoalo, 2800 m, 14 July 1960, *H. H. Iltis 224* (MICH, WIS). **Michoacán:** NNW of Ciudad Hidalgo & 2–3 km W of Cerro San Andres, 3200 m, 20 Mar. 1949, *R. McVaugh 9969* (GH, MICH, NY). **Morelos:** in mtns. along free Rte. 95, ca. 5 mi. S of Morelos–Federal District border, 14 Mar. 1961, *King 4154* (F, MICH, NY). **Oaxaca:** lumber rd. along ridge of Sierra, departing Hwy. 13 N of Ixtlan, 2800 m, 11 Jan. 1970, *Anderson 5414* (MICH, MO). **Puebla:** W-facing slope, 4100 m, 9 Feb. 1965, *E. S. Gibson 997* (F, KSC, MICH). **Sinaloa:** 2 air mi. NW of El Palmito, N of Hwy. 40, very near Durango state line, mpio. Concordia, Sierra Madre Occidental, ca. 105°51′W, 23°36′N, 2100 m, 27 Mar. 1981, *A. C. Sanders 4926* (KSC). **Tlaxcala:** above Hacienda de San Diego de Pinar, Mt. Malinche, 3100 m, 12 Dec. 1938, *Balls 5632* (F, GH, MICH). **Veracruz:** 1.3 km N of Vaqueria, 3 km by rd. from jct. Coscomatepec–Escola–Jacal rd., 14 km W of Escola, ca. 19°07′N, 97°14′W, 2900 m, 15 Nov. 1981, *M. Nee 23109* (F, WIS).

8. **Roldana cordovensis** (Hemsl.) H. Rob. & Brettell, Phytologia 27: 417. 1974. *Senecio cordovensis* Hemsl., Biol. Cent.-Amer., Bot. 2: 238. 1881. TYPE: Mexico. Veracruz: Fôret de la Trinidad Cordova, 7 Mar. 1866, *E. Bourgeau 2026* (holotype, K not seen; isotypes, F!, P not seen, P photos MICH!, MO!, P drawing GH!).

Senecio macrobotrys Hemsl., Biol. Cent.-Amer., Bot. 2: 243. 1881. Syn. nov. TYPE: Mexico. Veracruz: Orizaba, *Botteri 1103* (lectotype, designated here, K not seen; isotypes, GH!, G not seen, G photos F!, MICH!, MO!).

Cacalia peltata Sessé & Moc., Pl. Nov. Hisp. 132. 1887, nom. illeg., syn. nov., non *Cacalia peltata* Kunth, 1818; nec *Cacalia peltata* Rob. & Greenm., 1895. TYPE: Mexico, [s. loc.], *Sessé & Mociño 2819* (lectotype, designated here, F not seen, F photo MICH!).

Senecio chapalensis S. Watson, Proc. Amer. Acad. Arts 25: 155. 1890. Syn. nov. *Roldana chapalensis* (S. Watson) H. Rob. & Brettell, Phytologia 27: 416. 1974. TYPE: Mexico. Jalisco: Cañadas frias de boscaje, montañas ca. del Lago de Chapala, 22 Nov. 1892, *C. G. Pringle 2419* (holotype, GH!; isotypes, F!, MICH!, MO!, NY!).

Senecio brachyanthus Greenm., Ann. Missouri Bot. Gard. 1: 277. 1914. Syn. nov. TYPE: Mexico. Guerrero: betw. Ayusinapa & Petatlán, 1540–2155 m, *E. W. Nelson 2137* (lectotype, designated here, GH!; isotype, US not seen).

Senecio chapalensis var. *areolatus* Greenm., Ann. Missouri Bot. Gard. 1: 278. 1914. Syn. nov. TYPE: Mexico. Morelos: on shaded bluffs of a wet canyon above Cuernavaca, 1980 m, 15 Feb. 1899, *C. G. Pringle 8010* (lectotype, designated here, GH!; isotypes, F!, MICH!, MO!, NY!).

Shrublets to tree-like shrubs, 1–6 m tall; new growth pubescent with stipitate-glandular and multicelled hairs to glabrate; stems terete, green, red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 3–12 cm, glabrate to pubescent; blades 6–17(–22) × 7–15 (–22) cm, eccentrically peltate, triple-nerved, weakly palmatifid, base truncate, 5- to 7(to 15)-lobed, sinus depth about 1/4 of the way to the midrib, lobes acute-deltoid, margins weakly denticulate; abaxially weakly pubescent on veins and margins, rarely with a light tomentum of multicelled hairs, adaxially glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* pyramidal compound paniculate cyme, 10 to 30(to 50) capitula per branch, peduncles glabrate to densely pubescent with short stipitate-glandular hairs,

primary bracts sessile, broadly oval, 2–9 × 1–7 cm, ultimate peduncles 3–12 mm, bracteoles 2 to 3, filiform or linear, 2–3 mm. *Capitula* radiate or eradiate, 8–15 × 3–4 mm, funnelform, corollas yellow to whitish; calyculate bracts ca. 3, linear to filiform, 1–5(–10) mm; phyllaries (5 to)8, stipitate-glandular pubescent, 7–10 × ca. 1 mm. Ray florets absent or ca. 5, corolla ligulate or reduced to a tube, glabrous, tube 3–5 mm, ligule ca. 7 × 2 mm, or when reduced tube 2–3 mm. Disk florets 8 to 16, corolla funnelform, glabrous, 8–11 mm, tube 3–4 mm, throat 4–5 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, 1–2.5 mm, ribs 10, resin glands present; pappus bristles 7–9 mm.

Distribution and phenology. Central and southern Mexico (Aguascalientes, Durango, Guerrero, Jalisco, México, Michoacán, Morelos, Nayarit, Oaxaca, San Luis Potosí, Veracruz, Zacatecas) at elevations of 1500–2800(–3500) m. Found in pine-oak and fir forests, including shaded bluffs of wet canyons. Flowering November through February.

Comments. In the protologue for *Senecio macrobotrys*, Hemsley (1881) listed the syntypes *Bilimek 560* (K not seen, F!, F fragm. F!) and *Botteri 1103* (K, chosen here as lectotype), both from the same locality.

In the protologue for *Cacalia peltata* Sessé & Moc., Sessé and Mociño (1887) did not list any specimens; the lectotype is chosen from original Sessé & Mociño material at F.

Greenman (1914) listed *E. W. Nelson 2137* (GH, US) in the protologue as the type material for *Senecio brachyanthus*, and Greenman (1914) listed *C. G. Pringle 8010* (GH, MO) in the protologue as the type material for *S. chapalensis* var. *areolatus*. In both cases, the GH specimen was chosen as the lectotype.

Roldana cordovensis is a wide-ranging species. The morphology of its ray florets and leaf blades is variable. The ray florets may be completely absent from the capitula, reduced to tubes, or ligulate. While there appears to be a trend toward the reduction of ray florets in the southern range of the distribution, none of these forms can be correlated with a specific distribution. The shape of the leaf blade is highly variable; at times the blade has distinct acute lobes, and at others it has indistinct rounded lobes. There has also been some uncertainty as to whether the blade attachment is restricted to being eccentrically peltate, to the exclusion of having blades marginally attached to petiole. In my concept of this species, it is so restricted.

Roldana cordovensis was described by Hemsley (1881) as having eccentrically peltate blade attachment and ray corollas either with or without a ligule. He also described *Senecio macrobotrys* as essentially an eradiate *R. cordovensis*. However, as Gibson (1969) noted, the isotype has reduced rays present. *Senecio chapalensis* was described by Watson in 1890 as having eccentrically peltate leaves and radiate capitula. After comparing the type material available and numerous collections annotated under all three epithets, I have concluded that they are all within the fabric of *R. cordovensis*. Greenman (1901) proposed *S. chapalensis* var. *areolatus* to call attention to those plants with areolae on the undersurfaces of the leaf blade and with smaller rays. In comparing the type material of the variety with other collections of the species, there is no such difference; due to the high variability of the ray florets in this species, the variety is placed in synonymy. *Senecio brachyanthus* is a form of *R. cordovensis* from Guerrero with only multicelled trichomes as opposed to the more common condition of a combination of stipitate-glandular and multicelled trichomes. From my observations of the genus as a whole, these two types of trichomes are found in concert with varying densities, and the separation of a species based on this character alone is not tenable. *Cacalia peltata* Sessé & Moc. was described in 1887, and, after viewing a photograph of the type, it is placed in synonymy here.

The collections *Mexia 9055* and *G. B. Hinton 11320* both have notes by the collectors that they were white-flowered. Greenman (1926) noted that the rays of this species are whitish in the dried state. Due to the mixing of "cacalioid" and "senecionoid" characters in this genus, it is not surprising to see a variation in corolla color accompany the variation in ray floret morphology.

Representative specimens examined. MEXICO. **Aguascalientes:** ca. 10 mi. SE of Calvillo, 3 hours by horse from Rancho de Los Adobes, 2500 m, 4 Nov. 1959, *R. McVaugh 217* (MICH). **Durango:** 48 km WNW of Huejuquilla El Alto, Jalisco on rd. to Canoas, mpio. Mezquital, 2530 m, 21 Oct. 1983, *D. E. Breedlove 59127* (CAS). **Guerrero:** dist. Mina, second ridge N of Petlacala, 1900 m, 1 Jan. 1938, *Mexia 9055* (CAS, F, GH, MO, NY); ca. 2 km NE of Campamento El Gallo, ca. 17°28′N, 100°13′W, 2650 m, 27 Jan. 1965, *J. Rzedowski 132* (DS, MICH). **Jalisco:** ca. 31 rd. mi. W of Ayutla, ca. 70 mi. NW of Autlán, 2200 m, 3 Nov. 1962, *A. Cronquist 9797* (GH, KSC, MICH, MO, NY). **México:** cerca de Amecameca, 2500 m, 25 Feb. 1968, *J. Rzedowski 25440* (DS, MICH, WIS). **Michoacán:** Rincón, 1900 m, 1 Jan. 1911, *Arsene 6860* (MO); top of Bosques de San Francisco, ca. 10 km E of Ocampo (ca. 20 km NE of Zitácuaro), 19°35′N, 100°14′W, 3500 m, 19 July 1960, *H. H. Iltis 289* (LCC, WIS). **Morelos:** above Cuernavaca, 1980 m, 15 Feb. 1899, *C. G. Pringle 8010* (F, GH, MICH, MO, NY). **Nayarit:** Tepic, 8–9 km E of Camichin, 12 km from entrance of hwy. Tepic–Ixtlan, 21°27′N, 104°45′W, 1340 m, 11 Nov. 1993, *G. Flores-Franco 3263* (MO). **Oaxaca:** 17 km NE of Piedra Larga, rd. betw. Piedra Larga Miahuatlan, distr. of Juquila, 1260 m, 22 Nov. 1982, *Martínez S. 2750* (KSC, NY). **San Luis Potosí:** 22 km W of Santa Catarina on hwy. 86 at Km 49, 22°5′N, 100°40′W,

2200 m, 29 Sep. 1965, *Roe 2185* (WIS). **Veracruz:** 5.3 km W of Escola on rd. to Jacal, 18.5 km by rd. NW of Coscomatepec, mpio. Calcahualco. 19 10′N, 97 11′W, 2300 m, 12 Jan. 1981, *M. Nee 19719* (F, WIS). **Zacatecas:** ca. 25 km SW of Tlaltenango, 30 km W of the jct. Jalpa–Juchipila, 2550 m, 24 Jan. 1975, *R. McVaugh 25941* (MICH).

9. Roldana ehrenbergiana (Klatt) H. Rob. & Brettell, Phytologia 27: 418. 1974. *Senecio ehrenbergianus* Klatt, Leopoldina 24: 125. 1888. TYPE: same as that for *Senecio canicidus* Sessé & Moc. (neotype, designated here, MEXU not seen).

Senecio canicidus Sessé & Moc., Fl. Mexic. (ed 2) 185. 1894. Syn. nov. TYPE: Mexico. Puebla: *Sessé & Mociño s.n.* (neotype, designated here, MEXU not seen, MEXU photo MICH 1755!).

Senecio acerifolius Klatt, Ann. K. K. Naturhist. Hofmus. 9: 363. 1894, nom. illeg., syn. nov., non *Senecio acerifolius* K. Koch, 1861. TYPE: Mexico. [s. loc.], *Schmitz s.n.* (holotype, W not seen, W photos F!, MICH!, MO!).

Senecio semperamatae T. M. Barkley, Brittonia 30: 73. 1978. Syn. nov. TYPE: Mexico. México: in moist swale on S slope of volcanic ridge S of Mexico City, ca. 20 km NW of Cuautla, ca. 1300 m, 30 June 1974, *A. Cronquist 11130* (holotype, NY!; isotypes, CAS!, GH!, KSC!, MO!).

Small single-stemmed or clustered perennial herbs, 0.5–1 m tall, from a tuberous thickened fibrous rooted caudex, densely brown villous-lanate; plants glabrate to weakly pilose, patches of arachnoid pubescence at axils and under capitula, base of stem and caudex densely white-villous; stems terete, striated, maculate, mature stems reddish. *Leaves* cauline, evenly distributed on stem; petioles weak, 2–4 cm, sparsely pubescent; blades 5–10 × 4–7 cm, pinnately veined and lobed, 5 principal lobes, sinus depth more than 1/2 of the way to the midrib to incised, lobes weakly to strongly secondarily lobed, leaf base truncate to weakly cuneate and unequal, margin pubescent with multicelled hairs; abaxially glabrate with multicelled hairs on veins to pilose, adaxially glabrate to sparsely pilose with multicelled hairs along veins. *Capitulescence* variable from a simple panicle to a flat-topped corymbiform cyme, 1 to 2 capitula per branch, peduncles glabrate to pilose, primary bracts linear, 5–10 mm, ultimate peduncles 30–200 mm, bracteoles 0 to 2, filiform, up to 10 mm. *Capitula* radiate, 10–15(–22) × 5–10 mm, campanulate, corollas yellow; calyculate bracts filiform, ca. 5, 3–5 mm; phyllaries ca. 13, glabrate, 8–15 × 2.5–4 mm. Ray and disk corollas may have resin glands present. Ray florets 8, corolla ligulate, glabrous, tube ca. 3–5 mm, ligule 10–30 × 5–10 mm. Disk florets 50 to 60, corolla funnelform, glabrous, 7–13 mm, tube 2.5–5 mm, throat 3–5 mm, lobes 1–3 mm. Achenes glabrous, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles 5–10 mm.

Distribution and phenology. Central Mexico (México, Morelos, Puebla) at elevations of 1300–1600 m. Found in subtropical deciduous forests with *Prunus*, *Ipomoea*, *Ficus*, and *Mimosa*. Flowering May through June.

Comments. The holotype at Berlin for *Senecio ehrenbergianus* was destroyed in 1943: Mexico. Puebla: *C. Ehrenberg s.n.* (B photo MICH!, B drawing GH!). No other type material was found at HAL by Uwe Braun (pers. comm., Oct. 2004); therefore, the neotype is chosen from the senior synonym *S. canicidus* Sessé & Moc. In the protologue of *S. lobatus*, Sessé and Mociño (1894) did not list any specimens; the lectotype was chosen from original Sessé and Mociño material at MEXU.

Roldana ehrenbergiana is a distinctive perennial herb with large capitula and deeply lobed pinnate leaf blades. Barkley (1978) created the species *Senecio semperamatae* based on its having somewhat larger capitula and involucre 12–15 mm high versus 6–10 mm high. Due to the range of variation of involucre size that is present, one cannot base a separate species on this character alone. After examining the protologue and type material for both *S. acerifolius* Klatt and *S. canicidus* Sessé & Moc., they are placed in synonymy here.

Representative specimens examined. MEXICO. **México:** near México, 1893, *Ranirez s.n.* (GH). **Morelos:** Ejido Palpan, Km 20 Miacatlan–Tlanapalpan, mpio. Miacatlan, 1500 m, 5 June 1977, *García 338* (KSC). **Puebla:** Valsequillo, Arnica del Pams, 8 May 1966, *Boege 74* (CAS); Hierba del Perro, hierba de la Puebla, 8 May 1961, *Boege 561* (GH); vic. of San Luis Tultitlanapa, Puebla, near Oaxaca, 1 June 1908, *C. A. Purpus 2519* (CAS, F, GH, MO, NY).

10. Roldana eriophylla (Greenm.) H. Rob. & Brettell, Phytologia 27(6): 418. 1974. *Senecio eriophyllus* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 282. 1907. TYPE: Mexico. Oaxaca: hills near Tula, 20 May 1906, *C. G. Pringle 13864* (holotype, GH!; isotypes, GH!, MICH!).

Simple or sparingly branched shrubs or small trees, 2–3 m tall; new growth lanate, becoming glabrate; stems solid, terete, striated. *Leaves* seasonally deciduous, new leaves emanating from stem apex; petioles 3.5–5 cm, lanate to densely pubescent; blades 9–12 × 6–8 cm, palmate- to triple-nerved, ovate to oval, 7- to 11-lobed, sinus depth 1/8 or less of the way to the midrib, lobes deltoid, acute or rounded at apex, leaf base truncate to cuneate; abaxially variously lanate and canescent, adaxially glabrate to densely pubes-

cent canescent. *Capitulescence* pyramidal compound panicle with terminal cymes, 5 to 10 capitula per branch, peduncles lanate, primary bracts linear, 3–5 mm, ultimate pedicels 3–8 mm, bracteoles ca. 3, linear, ca. 2 mm. *Capitula* eradiate, 8–9 × ca. 3 mm, funnelform, corollas yellow; calyculate bracts ca. 3, linear, 2–3 mm; phyllaries 8, proximally lanate, distally glabrous, purple coloration along midrib, 6–7 × 1–2 mm. Disk florets 10 to 12, corolla slightly campanulate, glabrous, corolla 7–9 mm, tube 2–4 mm, throat 1–3 mm, lobes 2–4 mm, lobes longer than throat. Achenes glabrous, cylindrical, 1.5–2.5 mm, weakly 5-ribbed, resin glands absent; pappus bristles 6–7 mm.

Distribution and phenology. Southern Mexico (Chiapas, Oaxaca) at elevations of 1000–2300 m. Found in pine-oak forests, growing with *Quercus*, *Euphorbia* L., *Hauya* DC., *Diospyros* L., *Cedrela* P. Browne, *Trichilia* P. Browne, and *Heliocarpus* L. Flowering from May through October.

Comments. *Roldana eriophylla* has several features that make it unique: its corolla lobes are longer than the throat and the tomentose leaves are seasonally deciduous with new leaves emanating from the stem apex, creating a foreshortened stem. Both Bonnie Clark and José Luis Villaseñor recommend retaining it in *Roldana*, rather than transferring it to *Pittocaulon* or *Telanthophora* (pers. comm., July 2002).

Representative specimens examined. MEXICO. **Chiapas:** above El Chorreadero, mpio. of Ciapa de Corzo, *D. E. Breedlove 50639* (CAS); Monserrate, 1700 m, 1902, *C. A. Purpus 9000* (CAS). **Oaxaca:** Cerro San Filipe, 1700 m, 9 June 1906, *Conzatti 1366* (GH); 5 km SE of Gegoyache & 9 km SE of Totolapan, carr. Oaxaca–Tehuantepec, *Torres 2431* (TEX).

11. Roldana gentryi H. Rob. & Brettell, Phytologia 27: 418. 1974. *Senecio gentryi* (H. Rob. & Brettell) B. L. Turner & T. M. Barkley, Phytologia 67: 392. 1989. TYPE: Mexico. Sinaloa: above La Jolla, Sierra Surotato, pine-oak zone, 5000–6000 ft., 17–24 Mar. 1945, *H. S. Gentry 7178* (holotype, US not seen, US photos F!, GH!, MO!, NY!; isotypes, F!, GH!, NY!).

Senecio tepopanus B. L. Turner, Phytologia 74: 383. 1993. Syn. nov. *Roldana tepopana* (B. L. Turner) B. L. Turner, Phytologia 87: 248. 2005 [2006]. TYPE: Mexico. Sonora: Tepopa, Sierra Chiribo, Río Mayo drainage, "Upper Sonoran; dark canyon," 9 Mar. 1935, *H. S. Gentry 1411* (holotype, ARIZ not seen; isotypes, A!, CAS!, F!, MO 1089265!, MO 1094405!).

Shrubs, often tree-like, 2–3 m tall; new growth glabrate to densely pubescent with stipitate-glandular and multicelled hairs; stems terete, light green. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 6–13 cm, sparsely pubescent; blades 9–15 × 9–18 cm, palmate- to triple-nerved, shape various: cordate-deltoid with acute lobes; ovate, 5 to 7 shallow acute lobes; or palmatifid, 5 to 7 acute lobes, sinus depth about 1/4 of the way to the midrib; margins glabrous, weakly callous denticulate; abaxially glabrate with stipitate-glandular hairs along veins, adaxially glabrous. *Capitulescence* pyramidal compound paniculate cyme, 5 to 25 capitula per branch, peduncles sparsely to densely pubescent with stipitate-glandular and long multicelled hairs, primary bracts sessile, oval, 1.5–4 × 0.5–2 mm, or linear, 2–5 mm, ultimate peduncles 5–17 mm, bracteoles 2 to 4, linear to scale-like, ca. 2 mm. *Capitula* radiate, 12–15 × 3–5 mm, campanulate, corollas yellow; calyculate bracts 0 to 3, linear to scale-like, 1–2 mm; phyllaries 8, densely stipitate pubescent, purple-tinted, 7–9 × 1–2 mm. Ray florets 4 to 6, corolla ligulate, tube 5–6 mm, ligule 6–10 × 1.5–2 mm. Disk florets 6 to 12, corolla funnelform, glabrous, 9–10 mm, tube 2.5–4 mm, throat 4–5 mm, lobes 1–1.5 mm. Achenes glabrous, cylindrical, ca. 3 mm, ribs 10, resin glands absent; pappus bristles ca. 8 mm.

Distribution and phenology. Northern Mexico (Chihuahua, Durango, Jalisco, Sinaloa, Sonora) at elevations of (900–)1500–2700 m. Found in pine-oak forests. Flowering November through March.

Comments. *Roldana gentryi* most closely resembles *R. cordovensis*, but it differs by having marginally attached leaf blades and distribution restricted to northern Mexico.

Representative specimens examined. MEXICO. **Chihuahua:** E of La Bufa in ecotone betw. thorn forest & barrancan oak forest, mpio. Batopilas, Arroyo La Bufa, 107°35′W, 27°07′N, 900 m, 23 Mar. 1979, *R. A. Bye 9197* (UC). **Durango:** Sierra Madre Occidental, along hwy. 40 at the E end of El Espinazo de Diablo, 11.3 mi. SW of La Ciudad, 16 mi. E of Revolcaderos, ca. 105°50′W, 23°36′N, 2500 m, 26 Mar. 1981, *A. C. Sanders 4878* (KSC). **Jalisco:** 18 km despues de Bajio del Tule hacia Bolanos, mpio. Mezquitic, 12 Mar. 1991, *Cházaro B. 6579* (WIS). **Sinaloa:** 3 mi. NW of Los Hornos rd. to Mocorito, Sierra Suraotato, 2100 m, 25 Feb. 1969, *D. E. Breedlove 16529* (CAS, MICH); Sierra Tacuichamona, 1500 m, 18 Feb. 1940, *H. S. Gentry 5659* (GH, MICH, MO, NY). **Sonora:** Sierra Chiribo, Río Mayo, Upper Sonora, dark canyon, 9 Mar. 1935, *H. S. Gentry 1411* (A, CAS, F, MO).

12. Roldana gilgii (Greenm.) H. Rob. & Brettell, Phytologia 27: 419. 1974. *Senecio gilgii* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 282. 1907. TYPE: Mexico. Chiapas: near Pinabete, 6500–8000 ft., 8 Feb. 1896, *E. W. Nelson 3772* (lectotype, designated here, US not seen, US digital image US!; isotypes, GH!, MO!).

Suffruticose robust herbs, shrublets or shrubs, 1–4 m tall; new growth densely pubescent with stipitate-glandular and long multicelled hairs; stems terete, greenish brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 8–22 cm, densely pubescent; blades 10–30 × 10–30 cm, palmately nerved, rotund to reniform, 12(+)-lobed, sinus depth less than 1/4 of the way to the midrib, lobes acute, weakly secondarily lobed to irregularly serrate, margins pubescent with multicelled hairs; abaxially moderately to densely pubescent with stipitate-glandular and multicelled hairs, adaxially pubescent with stipitate-glandular hairs. *Capitulescence* pyramidal compound paniculate cyme, 5 to 25 capitula per branch, peduncles densely pubescent with a mixture of stipitate-glandular and long multicelled hairs, primary bracts linear, ca. 5 mm, ultimate peduncles 5–30 mm, bracteoles 2 to 6, linear, 2–4 mm. *Capitula* radiate, 12–17 × ca. 5 mm, campanulate, corollas yellow; calyculate bracts 1 to 3, linear, 2–3 mm; phyllaries 10 to 13, densely stipitate pubescent, 9–13 × ca. 2 mm. Ray florets 8 to 10, corolla ligulate, glabrous, tube ca. 5 mm, ligule 3–9 × 2–3 mm. Disk florets ca. 30, corolla weakly campanulate, glabrous, 9–10 mm, tube ca. 5 mm, throat 2–3 mm, lobes ca. 2 mm. Achenes glabrous, cylindrical, 3–4 mm, ribs 10, resin glands absent; pappus bristles 7–9 mm.

Distribution and phenology. Southern Mexico and Guatemala (Guatemala: Chimaltenango, Guatemala, Huehuetenango, Quezaltenango, Quiché, San Marcos, Sololá, Suchitepéquez, Totonicapán; Mexico: Chiapas) at elevations of 1600–3200 m. Found in montane rainforests. Flowering January through March.

Comments. Greenman (1907) cited *E. W. Nelson 3773* (GH, US, B) in the protologue; however, this appears to be a typographic error, as Greenman annotated, in hand, the *E. W. Nelson 3772* specimens from GH and US as the type collection. The Berlin specimen is presumed destroyed. Gibson selected the lectotype, in hand, in 1968.

The most distinguishing features of *Roldana gilgii* are the large rotund to reniform multilobed leaf blades and long brown multicelled trichomes.

Representative specimens examined. GUATEMALA. **Chimaltenango:** rd. to Panajachel betw. Los Idolos & Chocoyosm, 2400 m, 12–23 Jan. 1966, *Molina R 16189* (F). **Guatemala:** slopes of Volcán de Pacaya, betw. San Francisco Sales & the base of the active cone, 2000 m, 20 Dec. 1940, *P. C. Standley 80643* (F). **Huehuetenango:** about laguna de Ocubila, E of Huehuetenango, 1900 m, 7 Jan. 1941, *P. C. Standley 82668* (F). **Quezaltenango:** slopes of Volcán de Zunil, at & above Aguas Amargas, 2650 m, 17 Feb. 1939, *P. C. Standley 65297* (MO). **Quiché:** deep canyon in Sierra Madre Mtns. betw. Los Encuentros & Chichicastenango, 1900 m, 22 Dec. 1972, *Williams 41688* (F, MICH). **San Marcos:** betw. Todos Santos & Finca El Porvenir, lower to middle slopes of Volcán Tajumulco, 2300 m, 1 Mar. 1940, *J. A. Steyermark 37038* (F). **Sololá:** ravines near Nahuala, Sierra Madre Mtns., 2500 m, 17 Dec. 1962, *Williams 23187* (F, NY). **Suchitepéquez:** Volcán Atitlán, S side, 2200 m, 14 Jan. 1935, *A. F. Skutch 2131* (F, MO, NY). **Totonicapán:** rd. betw. San Francisco El Alto & Momostenango, 2600 m, 19 Jan. 1941, *P. C. Standley 84017* (F). MEXICO. **Chiapas:** on the SE side of Volcán Tacaná above Talquian, 2200 m, 5 Mar. 1972, *D. E. Breedlove 24411* (DS, MICH, MO).

13. Roldana glinophylla Gibson ex H. Rob. & Brettell, Phytologia 27: 419. 1974. *Senecio acerifolius* Hemsl., Biol. Cent.-Amer., Bot. 2: 235. 1881, nom. illeg., non *Senecio acerifolius* K. Koch, 1861. *Senecio glinophyllus* (H. Rob. & Brettell) McVaugh, Fl. Novo-Galiciana 12: 823. 1984. TYPE: Mexico. Michoacán or Oaxaca: "Oaxaca," *Ghiesbreght s.n.* (holotype, K not seen).

Simple or sparingly branched perennial herbs, ca. 1 m tall, plants glabrous; stems terete, finely striated, yellowish green. *Leaves* cauline, clustered at the midstem, reduced upward; petioles 3–4 cm, glabrous; blades 6–10 × 6–10 cm, papyraceous, palmately nerved, palmatifid, 5-lobed, sinus depth 1/2 to 1/4 of the way to the midrib, lobes acute, leaf base truncate, margins without callous denticles; surfaces glabrous. *Capitulescence* ascending simple to compound corymbiform cyme, sometimes giving an umbellate appearance, 3 to 8 capitula per branch, peduncles glabrous, primary bracts minutely foliaceous with 3 divaricate lobes, the lowermost secondarily lobed or linear, ca. 3 mm, ultimate peduncles 2–25 mm, bracteoles 0 to 3, linear, 4–7 mm. *Capitula* radiate, 10–15 × 3–5 mm, campanulate, corollas yellow; calyculate bracts 5 to 8, linear, 3–8 mm; phyllaries 13, glabrous, 10–11 × ca. 2 mm. Ray florets 5 to 8, corolla ligulate, glabrous, tube ca. 5 mm, ligule ca. 5 × 2.5 mm. Disk florets ca. 30, corolla funnelform, glabrous, ca. 8.5 mm, tube ca. 3.5 mm, throat ca. 3 mm, lobes ca. 2 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 6–7 mm.

Distribution and phenology. Mexico (Michoacán) at elevations of 1000–1500 m. Found in pine-oak forests. Collected in flower in September and October.

Comments. Hemsley (1881) lists *Ghiesbreght s.n.* (K) from southern Mexico, Oaxaca in the protologue. McVaugh (1984: 823) notes that he saw the *Ghiesbreght s.n.* (K) specimen, and that it is the type for both *Senecio acerifolius* Hemsl. and *S. glinophyllus*. He also notes that many of Ghiesbreght's duplicates were erroneously labeled from Oaxaca

when they were in fact from Michoacán or from other parts of Mexico. There is another set of possible type material at GH, a note by Greenman, 19 Sep. 1904, in hand, reads "*Ghiesbreght 371, Senecio acerifolius* Hemsl. verified at Kew with the original."

Roldana glinophylla is one of the glabrous perennial herbs found along the Trans-Mexican Volcanic Belt. The star-shaped leaf blade and corymbiform capitulescence make it unique.

Representative specimens examined. MEXICO. **Michoacán:** near Uruapan, terre temperee, *Ghiesbreght 371* (GH, MICH, NY); Coalcomán, distr. Coalcomán, *G. B. Hinton 12220* (GH, MICH, NY).

14. **Roldana gonzaleziae** (B. L. Turner) B. L. Turner, Phytologia 80: 278. 1996. *Senecio gonzaleziae* B. L. Turner, Phytologia 57: 377. 1985, as "gonzalezae." *Roldana gonzaleziae* (B. L. Turner) Funston, Novon 11: 304. 2001, as "gonzalezi," isonym. TYPE: Mexico. Durango: mpio. de Mezquital, 3 km S de Sta. Ma. de Ocotan, 17 Oct. 1984, *M. Gonzalez & S. Acevedo 1558* (holotype, TEX not seen, TEX digital image TEX!).

Perennial herbs, acaulescent, to 60 cm tall, arising from a fibrous rooted caudex, typically lanate; plants glabrate to sparsely tomentose; stems terete, striated, reddish. *Leaves* clustered at base; petioles 2–4 cm, pubescent to pilose; blades 5–7 × 5–9 cm, subpalmately nerved to triple-nerved, oval, weakly 8- to 13-lobed to crenate, margins pubescent; abaxially pilose to lanate pubescence, adaxially sparsely pubescent with glandular hairs. *Capitulescence* flat-topped corymbiform cyme, atop a 9.5–55 cm naked scape, ca. 15 capitula in the entire capitulescence, scape floccose tomentose, primary bracts linear, up to 5 mm, ultimate peduncles 0–10 mm, bracteoles 0 to 3, linear, 1–3 mm long. *Capitula* radiate, 7–9 × 2–3 mm, turbinate, corollas yellow; calyculate bracts ca. 3, linear, 1–2 mm; phyllaries 8, glabrous to arachnoid floccose, greenish, 5–6 × ca. 1 mm. Ray florets 5 to 8, corolla ligulate, glabrous, tube ca. 3 mm, ligule 4–6 × 2–3 mm. Disk florets 9 to 16, corolla funnelform, glabrous or minutely pubescent, 5–6.5 mm, tube 2.5–3 mm, throat 1–2 mm, lobes 1.5–2 mm. Achenes glabrous, cylindrical, ca. 1.5 mm, ribs 10, resin glands absent; pappus bristles 4–6 mm.

Distribution and phenology. North-central Mexico (Durango, Jalisco) at elevations of ca. 2700 m. Found in dry pine-oak forests. Collected in flower September and October.

Comments. Roldana gonzaleziae is one of the few small perennial herbs in the genus. It closely resembles *R. sundbergii* but is separated from it by having shorter scapes and a different form of capitulescence. They are separated geographically: *R. sundbergii* has only been collected in the Sierra Madre Oriental of Nuevo León, whereas *R. gonzaleziae* is found further south in the Trans-Mexican Volcanic Belt.

Representative specimens examined. MEXICO. **Durango:** mpio. de Mezquital, 3 km S de Sta. Ma. de Ocotan, 17 Oct. 1984, *M. Gonzalez & S. Acevedo 1558* (TEX digital image TEX). **Jalisco:** 74 km WNW of Huequilla El Alto, Jalisco near Canoas, mpio. Mezquital, 2700 m, 22 Oct. 1983, *D. E. Breedlove 59182* (CAS).

15. **Roldana greenmanii** H. Rob. & Brettell, Phytologia 27: 419. 1974. *Senecio greenmanii* (H. Rob. & Brettell) L. O. Williams, Phytologia 31: 441. 1975. TYPE: Guatemala. Santa Rosa: Pueblo Nuevo, Tetalhuleu, 800 m, "Mano de leon," edible, Mar. 1923, *R. Stricker 359* (holotype, US not seen, US photos F!, GH!, MO!, NY!).

Robust suffruticose herbs, shrubs or small weak trees, up to 8 m tall; new growth densely pubescent with stipitate-glandular and long multicelled hairs; stems terete, striate, green or red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 10–15 cm, pubescent; blades 7–30 × 10–25 cm, palmately or triple-nerved, orbicular, 9- to 13-lobed, sinus depth about 1/2 of the way to the midrib, lobes deeply cut producing almost rectangular segments topped with 3 acute lobes, upward on stem blades becoming subpalmatifid, margins always denticulate; abaxially pubescent with short and long multicelled hairs, densely so on veins, adaxially sparsely pubescent with short glandular hairs. *Capitulescence* a loose paniculate cyme, 7 to 20 capitula per branch, peduncles pubescent with stipitate-glandular and long multicelled hairs, primary bracts obovate to oval, up to 10 × 5 mm, ultimate peduncles 15–30 mm, bracteoles 2 to 3, linear, 2–3 mm. *Capitula* radiate or eradiate, 11–15 × 4–5 mm, cylindrical, corollas yellow; calyculate bracts absent or ca. 3, linear, ca. 2 mm; phyllaries ca. 8, sparsely stipitate pubescent, greenish purple-tipped, 9–11 × 1–2 mm. Ray florets absent or ca. 4, corolla inconspicuously ligulate, glabrous, tube ca. 4 mm, ligule ca. 3 × 0.5 mm. Disk florets 18 to 21, corolla narrow, glabrous, ca. 10 mm, tube ca. 5 mm, throat ca. 4 mm, lobes ca. 1 mm. Achenes glabrous, cylindrical, ca. 4 mm, ribs 10, resin glands absent; pappus bristles 7–9 mm.

Distribution and phenology. Southern Mexico and Guatemala (Guatemala: Alta Verapaz, Quezaltenango, San Marcos, Santa Rosa; Mexico: Chiapas) at elevations of 1800–2300 m. Found in montane cloud forests. Flowering February through April.

Comments. *Roldana greenmanii* is a very distinct species unlikely to be confused with any other. The rotund leaves are deeply cut, producing almost rectangular segments that are topped with three acute lobes. The capitulescence is open with delicate peduncles that have relatively few capitula.

Representative specimens examined. GUATEMALA. **Alta Verapaz:** vic. of Coban, 1300 m, Mar. Apr. 1941, *P. C. Standley 90921* (F). **Quezaltenango:** along Río Samala, near Santa María de Jesús, 1600 m, 25 Jan. 1941, *P. C. Standley 84525* (F). **San Marcos:** Barranca Eminencia, rd. betw. San Marcos & San Rafael Pie de la Cuesta, upper barranca betw. Finca La Lucha & Buena Vista, 2600 m, 6 Feb. 1941, *P. C. Standley 86357* (F). **Santa Rosa:** Pueblo Nuevo, Tetalhuleu, 800 m, "Mano de leon," Mar. 1923, *R. Stricker 359* (US photos F, GH, MO, NY). MEXICO. **Chiapas:** third ridge along logging rd. from las Margaritas to Campo Alegre, mpio. La Independencia, 2300 m, 18 Feb. 1973, *D. E. Breedlove 33710* (DS, F, MICH, MO, NY).

16. Roldana grimesii (B. L. Turner) C. Jeffrey, Kew Bull. 47: 55. 1992. *Senecio grimesii* B. L. Turner, Brittonia 40: 81. 1988. TYPE: Mexico. Hidalgo: 45 mi. N of Zimapán along hwy. 85, 14 Mar. 1983, *B. L. Turner 15088* (holotype, TEX not seen, TEX digital image TEX!; isotype, XAL not seen).

Senecio marquesii B. L. Turner, Brittonia 40: 83. 1988. Syn. nov. *Roldana marquesii* (B. L. Turner) C. Jeffrey, Kew Bull. 47: 55. 1992. TYPE: Mexico. Veracruz: Saltó del Gato, 4 km al este de Xalapa, 19°35′N, 96 33′W, ca. 1500 m, "Bosque caducifolio, secundaria, suelo rojizo profundo," 25 Apr. 1972, *W. Marquez R. 24* (holotype, TEX not seen, TEX digital image TEX!; isotypes, KSC!, MEXU not seen, TEX!, XAL not seen).

Senecio nesomiorum B. L. Turner, Phytologia 66: 467. 1989. Syn. nov. *Roldana nesomiorum* (B. L. Turner) C. Jeffrey, Kew Bull. 47: 55. 1992. TYPE: Mexico. Nuevo León: mpio. Doctor Arroyo, ca. 35 mi. NE of Doctor Arroyo, NE of San Antonio, Peña Nevada, N side of Cerro Peña Nevada, rd. ca. 2 mi. N of "El Puerto," 23 45′N, 99 52′W, 2650 m, 15 Sep. 1988, *G. Nesom 6712* (holotype, TEX not seen, TEX digital image TEX!; isotype, MEXU not seen).

Shrublets or shrubs, to 1.5 m tall; new growth greenish brown villous, covered with a mixture of short and long (up to 2 mm) glandular multicelled hairs; stems terete, bark dark brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 7–15 cm, pubescent with short and long multicelled hairs; blades ca. 18 × 22 cm, triple-nerved, ovate-orbicular, 9- to 13-lobed, sinus about 1/8 of the way to the midrib, lobes acute, weakly secondarily lobed to denticulate; abaxially hirsute to velutinous hirsute, margins pubescent, adaxially glabrate to weakly hirsute. *Capitulescence* compound paniculate cyme, 3 to 10 capitula per branch, peduncles villous, glandular, primary bracts broadly oblong, ca. 20 × 10 mm, ciliate margins of long multicelled hairs, hairs 1.5–2 mm, ultimate peduncles 30–35 mm, bracteoles elliptic-lanceolate, up to 20 mm, ciliate margins of long multicelled hairs. *Capitula* radiate or eradiate, 12–16 × 5–10 mm, campanulate, corollas yellow; calyculate bracts 3 to 6 in 1 or 2 apparent series, oblanceolate, 10–14 mm, glabrate, ciliate margins of long multicelled hairs; phyllaries 9 to 13, stipitate-glandular pubescent, margins ciliate, 10–11 × ca. 2 mm. Ray florets absent or (5 to)9 to 13, corolla ligulate or reduced to a tube, pubescent, tube 4–8 mm, ligule 9–10 × ca. 3 mm. Disk florets 25 to 65, corolla campanulate, pubescent, ca. 10 mm, tube ca. 5 mm, throat ca. 4 mm, lobes ca. 1 mm. Achenes glabrous or pubescent, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 8–10 mm.

Distribution and phenology. Western Mexico (Hidalgo, Nuevo León, Puebla, Veracruz) at elevations of 1500–2700 m. Found in pine-oak forests, scattered in an oak woodland in relatively level terrain. Flowering February through April.

Comments. *Roldana grimesii* is densely pubescent with both stipitate-glandular and long multicelled hairs. The pubescence is dark-purple to brown, somewhat like the pubescence found on *R. metepeca* (B. L. Turner) C. Jeffrey. The bracts of the capitulescence and the calyculate bracts are oblong-ovate with ciliate margins. The only other species in which this occurs, on occasion, is *R. angulifolia*.

Roldana grimesii was described from Hidalgo as having eradiate capitula and glabrous achenes. Turner (1989) described *R. marquesii* from Veracruz and separated it from *R. grimesii* based on its having radiate capitula and pubescent achenes. He further separated *R. nesomiorum* based on its having radiate capitula and glabrous achenes, from Nuevo León. Based on my examination of the collections at hand, the above correlation of characters does not exist. Achene pubescence is variable and may be related to maturity of the capitula and, while rays are either present or absent, there is no distributional relationship to this phenomenon. All three of these entities are alike with respect to their other morphological and habital characteristics; therefore, they are synonymized under the oldest name.

Representative specimens examined. MEXICO. **Hidalgo:** along hwy. 105 betw. Pachuca & Tampico, 27.2 mi. S of Huejutla, 1.2 mi. S of Quebradora, 0.9 mi. N of Santa María, 20 58′N, 98 34′W, 1315 m, 28 Feb. 1987, *T. B. Croat 65970* (KSC, MO). **Nuevo León:** mpio. Doctor Arroyo, ca. 35 mi. NE of Doctor Arroyo, NE of San Antonio, Peña Nevada, 23 15′N, 99 52′W, 2650 m, 15 Sep. 1988, *G. Nesom 6712* (TEX digital image TEX). **Puebla:** Apulco, 2 Apr. 1967, *Boege 536* (CAS). **Veracruz:** on hwy. 131, 13 mi.

from jct. with hwy. 141, 1500 m, 29 Apr. 1986, *LaFrankie 1078* (MO).

17. Roldana guadalajarensis (B. L. Rob.) H. Rob. & Brettell, Phytologia 27: 420. 1974. *Senecio guadalajarensis* B. L. Rob., Proc. Amer. Acad. Arts 26: 166. 1891. TYPE: Mexico. Jalisco: Lugares fertiles de la Barranca, cerca de Guadalajara, 10 Sep. 1890, *C. G. Pringle 3280* (holotype, GH!; isotypes, F!, GH!, GH fragm. & drawing GH!, MO!, NY!).

Single-stemmed perennial herbs, 1–2.5 m tall, arising from a woody caudex, plants essentially glabrous, stipitate-glandular hairs on leaf blades and occasionally with fine hairs on peduncles; stems terete, with rough vertical striations, reddish brown. *Leaves* cauline, clustered at midstem, reduced upward; petioles 1–3 cm, glabrous; blades 12–25 × 2–6 cm (making blade 7–10× longer than wide), pinnately veined, linear-oblong, margins serrate to only callous denticulate, stipitate-glandular hairs sometimes present; abaxially glabrous to having a covering of stipitate-glandular hairs on veins, pale, adaxially essentially glabrous. *Capitulescence* flat-topped compound corymbiform cyme, 5 to 30 capitula per branch, peduncles essentially glabrous, occasionally with tufts of fine hairs subtending the capitula, primary bracts linear up to 5 mm, ultimate peduncles 40–150 mm, bracteoles linear, 1–2 mm. *Capitula* radiate, 8–10 × 2–4 mm, campanulate, corollas bright yellow to golden; calyculate bracts ca. 5, linear, 2–3 mm; phyllaries ca. 12, glabrous, purple-tinted, 5–9 × ca. 1 mm. Ray florets ca. 5, corolla ligulate, glabrous, tube 3–5 mm, ligule 6–8 × 2–3 mm. Disk florets 16 to 19, corolla funnelform, glabrous, 6.5–9 mm, tube 2.5–4 mm, throat 2.5–3.5 mm, lobes ca. 1.5 mm. Achenes pubescent with fine hairs, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 4–6 mm.

Distribution and phenology. Western and central Mexico (Guanajuato, Jalisco, Michoacán, Nayarit) at elevations of 1000–2200 m. Oak-pine zone, on grazed and cut mountainsides, may be found on open woodlands, waste places, and grasslands. Associated with *Quercus*, *Pinus*, *Salvia*, and *Cercocarpus* Kunth. Flowering July through October.

Comments. *Roldana guadalajarensis* is one of the few species with pinnately veined leaves in the genus. The leaves are further distinguished by being typically 7–10× longer than wide. This species is also one of the few possessing a flat-topped corymbiform cyme for its capitulescence type.

Representative specimens examined. MEXICO. **Guanajuato:** De Moro Leon, 10 July 1900, *Duges 1003* (GH). **Jalisco:** 32 km NW de Colima, 2–3 km SW of El Terreo, 19°25′50″N, 103°50′00″W, 2100 m, 22 Sep. 1987, *Cuevas 2480* (WIS). **Michoacán:** grazed woodland N of Los Reyes, 1200 m, 28 Oct. 1962, *R. McVaugh 21962* (MICH). **Nayarit:** along unused rd. from Mesa del Nayar to Santa Teresa, mpio. El Nayar, 1800 m, 12 Aug. 1980, *D. E. Breedlove 45420* (CAS).

18. Roldana hartwegii (Benth.) H. Rob. & Brettell, Phytologia 27: 420. 1974. *Senecio hartwegii* Benth., Pl. Hartw. 18. 1839. TYPE: Mexico. Jalisco: In sylvis Bolaños, 1839, *Hartweg 124* (holotype, K not seen, K photos F!, MICH!; isotypes, GH!, NY!).

Senecio seemannii Sch. Bip. in Seem., Bot. Voy. Herald 311. 1856. TYPE: Mexico. [state unknown; according to Seemann's travel agenda, this collection came from either western Durango, adjacent Sinaloa, or Nayarit]: Sierra Madre, *B. Seemann 2010* (holotype, GH!; isotypes, K not seen, P not seen, K photos F!, MICH!).

Cacalia tepicana M. E. Jones, Contr. W. Bot. 15: 156. 1929. TYPE: Mexico. Nayarit: Tepic, 10 Feb. 1927, *Marcus E. Jones 23357* (holotype, POM not seen; isotype, US!).

Roldana pennellii H. Rob. & Brettell, Phytologia 27(6): 422. 1974. Syn. nov. *Senecio octobracteatus* B. L. Turner & T. M. Barkley, Phytologia 67: 392. 1989, non *Senecio pennellii* Greenm., 1923. TYPE: Mexico. Durango: Barranca below Sandia Station, 7000 ft., 12 Oct. 1905, *C. G. Pringle 13568* (holotype, US not seen, US photos F!, GH!, MO!, NY!; isotypes, CAS!, GH!, MICH!, MO!, NMC!).

Single-stemmed perennial herbs, 1–2 m tall, arising from a woody caudex with grey-brown woolly buds and/or with branching rhizomes; new growth arachnoid pubescent; stems terete or angulate, purplish red or green, striate or maculate. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 3–7 cm, glabrous to sparsely arachnoid pubescent; blades 8–15 × 8–17 cm, palmate- to triple-nerved, oval to ovate, 9- to 15-lobed, sinus depth about 1/4 of the way to the midrib, lobes acute, weakly secondarily lobed to irregularly serrate-denticulate or rarely orbicular and subentire; abaxially glabrous to sparsely arachnoid pubescent on veins, rarely densely arachnoid pubescent, adaxially glabrous. *Capitulescence* compound paniculate (rarely corymbiform) cyme, 10 to 30(to 50) capitula per branch, peduncles sparsely arachnoid pubescent, primary bracts linear, 1–3 mm, ultimate peduncles 3–10 mm, bracteoles 0 to 3, linear, 1–2 mm. *Capitula* radiate or eradiate, 6–10 × ca. 4 mm, campanulate, corollas yellow; calyculate bracts 1 to 3, linear, 1–2 mm; phyllaries (5 to)8 to 13, glabrous to sparsely arachnoid pubescent, greenish purple-tipped, 4–7 × 1–2 mm. Ray florets absent or (3 to)5 to 8, corolla ligulate, pubescence of glandular hairs on tube, tube ca. 4(–5) mm, ligule ca. 4(–6) × 2–3 mm. Disk florets 6 to 12, corolla funnelform, pubescence of glandular

hairs on tube, ca. 8 mm, tube ca. 4 mm, throat ca. 3 mm, lobes ca. 1 mm. Achenes glabrous or pubescent, cylindrical, 1–2 mm, ribs 10, resin glands absent; pappus bristles 4–6 mm.

Distribution and phenology. Southwestern United States, northern and west-central Mexico, southern Mexico (Mexico: Chiapas, Chihuahua, Coahuila, Durango, Jalisco, Nayarit, Nuevo León, Oaxaca, Sinaloa, Sonora; United States: Arizona and New Mexico) at elevations of 1200–2700 m. Found in pine-oak forests and thickets near streams. Flowering August through November.

Comments. In the protologue for *Senecio hartwegii*, Bentham (1839) does not list any specimens; however, Hemsley (1881: 241) lists three specimens from Kew in his treatment of *Senecio*, including *Seemann 2010*, *Gregg 622*, and *Hartweg 124*. It is presumed that Hemsley would be examining Bentham's type material; based on this and the K photographs, the lectotype is chosen here.

Roldana hartwegii is a common species along the northern mountain chains and western end of the Trans-Mexican Volcanic Belt of Mexico. In gross morphology, it is consistent and easily recognizable. However, there has been disagreement over the significance of the variation found in the number of phyllaries, pubescence of the achenes, and morphology of the stems. Upon performing a numerical analysis of the complex on 64 collections (see Materials and Methods section), I have concluded that there is a recognizable trend in the correlation of these characters. Plants with 10 to 13 phyllaries and pubescent achenes are distributed along the Pacific slopes of the Sierra Madre Occidental and Trans-Mexican Volcanic Belt; plants with ca. 8 phyllaries and pubescent or glabrous achenes are distributed on the inner slopes of the Sierra Madre Occidental and Trans-Mexican Volcanic Belt, and on the northern end of the Sierra Madre Oriental; lastly, plants with 5 phyllaries and pubescent or glabrous achenes are seemingly restricted to southern Durango. Concolorous stems occur in steppe and desert climates, while maculate stems occur in tropical and midlatitude rainy climates. Angulate stems are associated with tropical rainy climates, while terete stems are associated with the midlatitude rainy climates and steppe and desert climates.

Over the years, several authors have recognized this variation by circumscribing new species, but in gross morphology and general habit, all of the collections examined are deemed to be the same species.

Roldana hartwegii was originally described by Bentham in 1839 as having stems floccose to arachnoid tomentose, leaves glabrous adaxially and white pubescent abaxially, 6 to 8 phyllaries, 3- to 4-rayed, 8 to 10 disks, and achenes puberulent. Having examined the protologues and type material for *Senecio seemannii* and *Cacalia tepicana*, they are easily placed in synonymy here, as was done by McVaugh (1984). Robinson and Brettell (1974) described *R. pennellii* as having eight phyllaries, glabrous achenes, concolorous stems, and distribution in the interior of Mexico. In general, these characters occur within *R. hartwegii* as circumscribed here; achene pubescence in this region is quite variable, and therefore does not warrant taxonomic recognition.

Key to the Varieties of *Roldana hartwegii*

1a. Phyllaries 5; plants from southern Durango . . . . . 18b. *R. hartwegii* var. *durangensis*
1b. Phyllaries 6 to 8 or phyllaries 10 to 13.
  2a. Phyllaries 10 to 13; plants from the Sierra Madre Occidental . . . . . 18c. *R. hartwegii* var. *carlomasonii*
  2b. Phyllaries 6 to 8.
    3a. Phyllaries ca. 5 × 1 mm; abaxial leaf pubescence glabrate; plants found in the southwestern United States, north and west-central Mexico . . . . . 18a. *R. hartwegii* var. *hartwegii*
    3b. Phyllaries ca. 7 × 2 mm; abaxial leaf pubescence persistent; plants found in Oaxaca and Chiapas . . . . . 18d. *R. hartwegii* var. *subcymosa*

18a. **Roldana hartwegii** var. **hartwegii**

Distinguishing characters. Stems typically terete, occasionally angulate, concolorous or maculate; leaves abaxially glabrate; phyllaries 6 to 8, 4–5.5 × ca. 1 mm; achenes pubescent or glabrous.

Distribution. Primarily along the inner slopes of the Sierra Madre Occidental and northern end of the Sierra Madre Oriental: Chihuahua, Coahuila, Durango, Jalisco, Nayarit, Nuevo León, Sinaloa, Sonora, Zacatecas.

Representative specimens examined. MEXICO. **Chihuahua:** Tarahumara sect. of Sierra Madre Occidental, ca. 5 km NE of Memelichi, & ca. 20 km SE of Cascada de Basaseachie, 2200 m, 25 Aug. 1980, *A. Cronquist 11761* (NY). **Coahuila:** northern Coahuila, higher elevations in the Sierra Jardin, 2800 m, 1 Sep. 1966, *Flyr 1182* (MO); middle & upper reaches of Cañon de la Hacienda, S of Rancho Cerro de la Madera, N slope of Sierra de la Madera, 27°3–4′N, 102 25′W, 2000 m, 21 Sep. 1972, *Chiang 9452a* (TEX). **Durango:** steep slopes at base of Espinosa del Diablo, 4 km NW of Los Angeles rd. betw. Mazatlán & Durango, 2500 m, 28 Oct. 1973, *D. E. Breedlove 35753* (CAS, MO); Sierra Madre Occidental, ca. 10 mi. W of El Saltó, 2900 m, 2 Oct. 1962, *A. Cronquist 9587* (G, KSC, MICH, MO, NY). **Jalisco:** mpio. Bolanos, 25 km W of Bolanos, camino a Tuxpan & Huicholes, 2530 m, 19 Oct. 1983, *Lott 2064* (KSC, MICH). **Nayarit:** 9.6 km N of Mesa de Nayar, rd. betw. Cienega Santa Teresa, 22 15′N, 104°40′W, 6 Nov. 1988, *O. Tellez V. 11590* (MEXU, MO). **Nuevo León:** Sierra Madre Oriental,

La Encautada, 23°55′40″N, 99°50′50″W, mpio. Gal I. Zaragosa, 2250 m, 28 Aug. 1975, *Robert s.n.* (MICH). **Sinaloa:** on small dirt logging rd. near Loberas Microwave Station, mpio. Concordia, 2000 m, 18 Oct. 1983, *D. E. Breedlove 58848* (CAS). **Sonora:** region of the Río de Bavispe, NE Sonora, El Picacho del Pilar, 2200 m, 13 Oct. 1941, *White 4818* (MICH). **Zacatecas:** ca. 38 km W of Jalpa, sobre la carretera Tlaltenango, 30 km del entronque con la carretera Jalpa–Juchipila, 2550 m, 21 Oct. 1973, *J. Rzedowski 939* (MICH, MO, NY, OS).

18b. Roldana hartwegii var. **durangensis** (H. Rob. & Brettell) Funston, comb. nov. Basionym: *Roldana pennellii* var. *durangensis* H. Rob. & Brettell, Phytologia 27: 423. 1974. *Senecio octobracteatus* var. *durangensis* (H. Rob. & Brettell) B. L. Turner & T. M. Barkley, Phytologia 67: 392. 1989. TYPE: Mexico. Durango: El Saltó, Sierra Madre Occidental, rocky, andesitic, pineland canyon, 2500–2530 m, herb, flowers yellow, 31 Aug. 1934, *F. W. Pennell 18500* (holotype, US not seen; isotypes, GH!, NY!).

Distinguishing characters. Stems terete (rarely angulate), concolorous (rarely maculate); leaves abaxially glabrate; phyllaries 5, 4–5.5 × ca. 1 mm; achenes pubescent or glabrous.

Distribution. Southern Durango, Mexico.

Comments. Robinson and Brettell (1974) described variety *durangensis* from the interior of southern Durango as having five phyllaries and glabrous achenes. In the eight collections I examined, five had glabrous achenes and three were pubescent. Regarding distribution, all eight were distributed near El Saltó, many to the west and seemingly on the slopes of the Sierra Madre Occidental. Due to the restricted distribution of five phyllaried plants in southern Durango, the taxon is being maintained as a variety, although within the species *Roldana hartwegii*.

Representative specimens examined. MEXICO. **Durango:** 54 mi. N of Estatión Coyotes along a lumber rd., just NW of Guachichilas, 3000 m, 5 Nov. 1970, *D. E. Breedlove 18788* (CAS); Puerto Buenos Aires, 8 km E of La Ciudad, 53 km W of El Saltó, rd. Durango–Mazatlán, 2650 m, 8 Nov. 1978, *García 898* (KSC, MO); 24 mi. E of El Saltó, ca. 3 mi. W of Los Navios, 23 Oct. 1980, *Spellenberg 1541* (NMC); 3 mi. W of El Saltó, 11 Aug. 1956, *Waterfall 12671* (MICH).

18c. Roldana hartwegii var. **carlomasonii** (B. L. Turner & T. M. Barkley) Funston, comb. nov. Basionym: *Senecio carlomasonii* B. L. Turner & T. M. Barkley, Phytologia 67: 390. 1989. *Roldana carlomasonii* (B. L. Turner & T. M. Barkley) C. Jeffrey, Kew Bull. 47: 54. 1992. TYPE: Mexico. Sonora: "along the road betw. Yapachic & Yecora, 19 mi. W of border with Chihuahua, dry, rocky hillside, with oaks," 24 Sep. 1984, *S. Sundberg 2839* (holotype, TEX not seen, TEX digital image TEX!; isotypes, CAS!, MEXU not seen).

Distinguishing characters. Stems terete or angulate, typically maculate, occasionally concolorous; leaves abaxially glabrate; phyllaries 10 to 13, 4–5.5 × ca. 1 mm; achenes pubescent.

Distribution. Primarily along the Pacific slopes of the Sierra Madre Occidental: Chihuahua, Jalisco, Nayarit, Sinaloa, Sonora.

Comments. Turner and Barkley (1989) described the species *Senecio carlomasonii* based on the following: 10 to 13 phyllaries, pubescent achenes, either concolorous or maculate stem color, and a distribution from Nayarit northward to southern Arizona. Their concept of *Roldana hartwegii* had a distribution restricted to southern Durango, Nayarit, and adjacent Jalisco. My treatment of the species distribution emphasizes an east-west trend rather than a north-south one, placing the variety *carlomasonii* on the Pacific slopes of the Sierra Madre Occidental.

Representative specimens examined. MEXICO. **Chihuahua:** Pinas Altos, S wall of Verde Arroyo, 28°16′30″N, 108°18′00″W, 2100 m, 21 Oct. 1945, *Hewitt 59* (GH). **Jalisco:** Volcán Tequila, rd. to microwave station, ca. 20°47′N, 103°50′W, 1600 m, 25 Oct. 1970, *Webster 15963* (DS, MICH). **Nayarit:** along rte. 28, near Villa Caranza, ca. 5 mi. from jct. of hwy. 15 & hwy. 28, along hwy. from Tepic to Jalcacatlán, 7 Jan. 1979, *T. B. Croat 45137* (KSC, MO). **Sinaloa:** along a small stream 0.5 mi. N of Los Ornos, mpio. Badiraguato, 1600 m, 1 Nov. 1969, *D. E. Breedlove 16765* (CAS, MICH); hillside 18 mi. NE of town 6.5 mi. NE of El Cajon, 26°N, 108°W, 1400 m, 26 Nov. 1975, *Lehto 19526* (MICH, NY, OS). **Sonora:** Sierra de Alamos, 1100 m, 6 Nov. 1939, *H. S. Gentry 4902* (DS, GH, MO, NY); along the rd. betw. Yapachic & Yecora, 19 mi. W of the border with Chihuahua, 24 Sep. 1984, *S. Sundberg 2839* (CAS).

18d. Roldana hartwegii var. **subcymosa** (H. Rob.) Funston, comb. nov. Basionym: *Roldana subcymosa* H. Rob., Phytologia 32: 332. 1975. *Senecio subcymosus* (H. Rob.) B. L. Turner & T. M. Barkley, Phytologia 67: 392. 1989. TYPE: Mexico. Oaxaca: in a moist ravine in pine-oak-alder zone, Sierra Madre del Sur, ca. 125 km S of Oaxaca, on rd. to Puerto Angel, ca. 2400 m, 7 Nov. 1970, *A. Cronquist & J. J. Fay 10888* (holotype, US not seen; isotypes, CAS!, F!, GH!, KANU!, MICH!, NY!).

Senecio tonii B. L. Turner, Phytologia 71: 304. 1991. *Roldana tonii* (B. L. Turner) B. L. Turner, Phytologia 87: 249. 2005 [2006]. TYPE: Mexico. Chiapas: mpio. San Cristóbal de las Casas, Santa Cruz en San Felipe, 15 Nov. 1986, *A. Mendez Ton 9481* (holotype, TEX!).

Distinguishing characters. *Leaf* blades abaxially lightly tomentose to persistently arachnoid pubescent

on veins and veinlets, adaxially glabrescent. *Capitulescence* compound paniculate cyme, 30 to 50 capitula per branch. *Capitula* radiate, ca. 9 × 3 mm; phyllaries ca. 8, glabrous to arachnoid, ca. 7 × 2 mm. Ray florets ca. 3, corolla ligulate, tube pubescent or glabrous, tube ca. 5 mm, ligule ca. 6 × 2 mm. Disk florets ca. 7, pubescent or glabrous. Achenes pubescent.

Distribution and phenology. Southern Mexico (Chiapas, Oaxaca) at elevations of 2400 m. Found in pine-oak forests. Flowering in November.

Comments. *Roldana hartwegii* var. *subcymosa* is the southern-most extension of *R. hartwegii*. Due to its denser pubescence, larger phyllaries, the occasional glabrosity of the corollas, and the geographic separation between the southern boundary of the typical variety and variety *subcymosa*'s distribution, it is being given varietal rank. Based on my examination of the protologue and type material, *Senecio tonii* is placed in synonymy here.

Representative specimens examined. MEXICO. **Chiapas:** on Cerro Huitepec (Muk'ta vits) W of San Cristóbal de las Casas, 2700 m, 5 Dec. 1971, *D. E. Breedlove 23034* (DS, MICH, MO, NY); forest near summit Cerro Zontehuitz, mpio. of San Cristóbal de las Casas, 2800 m, 17 Nov. 1981, *D. E. Breedlove 55611* (CAS); below Zinacantan Center along the trail to Ixtapa, 6200 ft., 5 Dec. 1966, *R. M. Laughlin 2929* (DS, MICH). **Oaxaca:** Sierra Madre del Sur, ca. 125 km S of Oaxaca, on the rd. to Puerto Angel, 2400 m, 7 Nov. 1970, *A. Cronquist & Fay 10888* (CAS, F, GH, KANU, MICH, NY).

19. Roldana hederifolia (Hemsl.) H. Rob. & Brettell, Phytologia 27: 420. 1974. *Senecio hederifolius* Hemsl., Biol. Cent.-Amer., Bot. 2: 241. 1881, as "hederaefolius." *Cacalia hederifolia* (Hemsl.) A. Gray ex Urbina, Cat. Pl. Mexic. 188. 1897. TYPE: Mexico. Oaxaca: from Monte Pilado & on Canetzeq, NE from Oajaca, Feb. 1845, *Jurgensen 379* (holotype, K not seen; isotypes, G not seen, K fragm. & drawing GH!, G photos F!, MICH!).

Perennial suffruticose (scandent?) herbs, 1–3 m tall; new growth glabrate to sparsely stipitate-glandular pubescent(?); stems terete, yellowish. *Leaves* cauline, evenly distributed on upper half of stem; petioles ca. 10 cm; blades 7.5–10 × 7.5–10 cm, triple-nerved, subhastate, 3 primary lobes, lowermost weakly secondarily lobed or subcordate with 5 weak lobes(?). *Capitulescence* a lax compound corymbose panicle, 3 to 10 capitula per branch, primary bracts lanceolate. *Capitula* eradiate, narrowly campanulate; phyllaries ca. 8, slightly pubescent; disk florets ca. 20, corolla funnelform, glabrous; achenes glabrous, pappus about equal in length to corolla. (Description compiled from the literature.)

Distribution. Mexico (Oaxaca) at elevations of ca. 2000 m. Found in pine-oak forests.

Comments. *Roldana hederifolius* as described by Hemsley (1881) has leaf blades marginally attached to the petiole and discoid capitula, with the type collection from the state of Oaxaca. It may ultimately fall into synonymy under *R. aliena*.

20. Roldana heracleifolia (Hemsl.) H. Rob. & Brettell, Phytologia 27: 420. 1974. *Senecio heracleifolius* Hemsl., Biol. Cent.-Amer., Bot. 2: 241. 1881. TYPE: Mexico. [east-central Mexico], Guadalupe valley, 7 Oct. 1869, *Bilimek 558* (lectotype, designated here, K not seen; isotypes, NY!, P not seen).

Large single-stemmed perennial herbs or subshrubs, 1–3 m tall, arising from a lanate woody caudex, new growth glabrate with tufts of arachnoid hairs; stems terete, striate, maculate, brown to red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous on shrubs or clustered at midstem on herbs; petioles 5–10 cm, glabrate with tufts of arachnoid hairs at axils; blades 15–30 × 15–20 cm, pinnately veined and lobed to dissected, typically 7-lobed, sinus depth 3/4 of the way to the midrib or incised (larger leaves may be condensed so as to appear ovate and deeply palmately lobed, yet the venation is still pinnate), lobes acute to obtuse, weakly secondarily lobed to irregularly serrate or merely callous denticulate, margin weakly pubescent, leaf base weakly auriculate with a winged petiole; adaxially glabrate, sparsely to densely pubescent on veins, abaxially glabrous. *Capitulescence* ascending compound corymbiform cyme, 10 to 30 capitula per branch, peduncles glabrous with arachnoid tufts at axils, primary bracts linear, up to 5 mm, ultimate peduncles 3–10 mm, bracteoles 2 to 3, linear, 1–3 mm. *Capitula* radiate, 8–12 × 2–3 mm, narrowly campanulate, corollas bright yellow; calyculate bracts ca. 3, linear, 1–2 mm; phyllaries ca. 8, glabrous to slightly pubescent, sometimes purple-tinted, 6–8 × ca. 2 mm. Ray florets 5 to 7, corolla ligulate, glabrous, tube ca. 5 mm long, ligule 5–7 × 2–3 mm. Disk florets 12 to 16, corolla funnelform, glabrous, ca. 8 mm, tube ca. 3 mm, throat ca. 3 mm, lobes ca. 2 mm. Achenes pubescent with short hairs along the ribs, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles 5–7 mm.

Distribution and phenology. Central Mexico (Aguascalientes, Guanajuato, Jalisco, México, Michoacán, Querétaro, Zacatecas) at elevations of 1600–2700 m. In oak forests and open disturbed woodlands, roadsides, pastures. Flowering September through November.

Comments. Hemsley (1881) lists two specimens from K in the protologue: S Mexico, Guadalupe, valley of Mexico, *Bilimek 558*; and, without locality, *Mackenzie s.n.* The *Bilimek 558* (K) specimen is chosen as the lectotype. As McVaugh (1984: 826) notes, the exact location from which the type was collected is an enigma, as there is no modern record of its occurrence in or about Guadalupe.

Roldana heracleifolia resembles *R. lineolata*; however, it grows slightly taller, typically has more disk florets per capitula, and has pubescent achenes. While both have pinnately lobed leaf blades, *R. heracleifolia*'s sinus depth is more than 1/2 of the way to the midrib and its blades are abaxially glabrate with veins pubescent, whereas *R. lineolata*'s sinus depth is typically only 1/3 or less of the way to the midrib and its blades are abaxially tomentose.

Representative specimens examined. MEXICO. **Aguascalientes:** Mesa de Prenadas Palo Alto, 2400 m, 23 Sep. 1980. *Gutierrez 111* (KSC). **Guanajuato:** mpio León, 13 km N of León, about El Puero, 2200 m, 30 Oct. 1988, *R. & J. D. Galván 3214* (MO). **Jalisco:** vic. of Paso de la Troje, rd. from Ojuelos to Aguascalientes, roadside ditch near Km 39, 2 mi. W of pass, 2200 m, 1 Nov. 1959, *R. McVaugh 8* (MICH, NY). **México:** Tlalpujahua, 2700 m, 1 Oct. 1908, *Diguet s.n.* (MICH). **Michoacán:** at a small summit ca. 6 mi. E of Morelia, 2200 m, 9 Oct. 1965, *A. Cronquist 10304* (CAS, GH, KANU, KSC, MICH, NY, WIS). **Querétaro:** rd. along SLP & San Miguel de Allende, ca. Km 8, 2150 m, 3 Oct. 1976, *Arguelles 609* (CAS). **Zacatecas:** Km 18 de la brecha Villanueva–El Plateado, mpio. Villanueva, 2000 m, 5 Nov. 1978, *García 866* (CAS, DAV, F, MICH, MO).

21. Roldana heterogama (Benth.) H. Rob. & Brettell, Phytologia 27: 420. 1974. *Cacalia heterogama* Benth. in Örst., Vidensk. Meddel. Dansk Naturhist. Foren. Kjobenhavn 1852(5–7): 107. 1853. *Senecio heterogamus* (Benth.) Hemsl., Biol. Cent.-Amer., Bot. 2: 242. 1881. TYPE: Costa Rica. Cartago Province: Volcán de Irazu, [1845], *A. S. Örsted 192* (lectotype, designated here, K not seen; isotypes, C 8858 not seen, microfiche IDC 2204. 190 III 4–5!, K fragm. & drawing GH not seen, C photos F!, GH!, MO!, NY!).

Senecio heterogamus var. *kellermanii* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 282. 1907. Syn. nov. TYPE: Guatemala. Sacatepéquez: Volcán de Aqua, 9100 ft., 15 Feb. 1905, *W. A. Kellerman 4706* (holotype, F!).

Cacalia calotricha S. F. Blake, Contrib. Gray Herb. 52: 58. 1917. Syn. nov. TYPE: Guatemala. Sacatepéquez: Volcán de Aqua, above Santa María de Jesús, 7500 ft., 4 Mar. 1916, *E. W. D. Holway 570* (holotype, GH not seen, GH photo MICH!, GH drawing NY!).

Suffruticose herbs, shrublets or shrubs, 1–5 m tall; new growth glabrous to densely pubescent with stipitate-glandular and long multicelled hairs; stems terete, dark brown. *Leaves* cauline, about evenly distributed on upper half of stem, lowermost deciduous; petioles 8–16 cm, pubescent with short and long multicelled hairs; blades 8–20(30–35) × 8–20(30–35) cm, principal leaves eccentrically peltate, triple-nerved, ovate-orbicular, 7- to 11-lobed, sinus depth about 1/4 of the way to the midrib, lobes acute, secondarily lobed, margins callous denticulate, pubescent; abaxially pubescent with stipitate-glandular hairs, densely so on veins with long multicelled brown hairs, adaxially sparsely pubescent with short glandular hairs. *Capitulescence* a loose compound paniculate cyme, 6 to 20 capitula per branch, peduncles pubescent with a mixture of stipitate-glandular and long multicelled hairs, primary bracts obovate to oblanceolate, 10–30 × 3–10 mm, ultimate peduncles 10–30 mm, bracteoles 1 to 3, linear, 3–6 mm. *Capitula* eradiate, (5–)9–15 × 3–5 mm, narrowly campanulate, corollas yellow; calyculate bracts ca. 3, linear 2–6 mm; phyllaries 8 to 13, stipitate-glandular pubescent, greenish purple-tinted, 8–13 × ca. 1 mm. Disk florets (17 to)20 to 40, corolla campanulate, glabrous, ca. 8 mm, tube ca. 3 mm, throat ca. 3 mm, lobes ca. 2 mm. Achenes glabrous, cylindrical, ca. 1 mm, ribs 10, resin glands absent; pappus bristles ca. 7 mm.

Distribution and phenology. Southern Mexico to northern Panama (Costa Rica: Cartago, Puntarenas, San José; Guatemala: Chimaltenango, Guatemala, Huehuetenango, Quezaltenango, Quiché, Sacatepéquez, San Marcos, Sololá, Totonicapán; Mexico: Chiapas, Oaxaca; Panama: Bocas del Toro, Chiriquí) at elevations of 3000–4100 m. In pine-oak forests, mostly on upper volcanic slopes. Flowering December through April.

Comments. In the protologue, Bentham (Örsted, 1853) listed type material from "Irasu, 8000 ft." but did not list any specimens. Hemsley (1881: 242) listed *A. S. Örsted 192* (K) when he made his combination in *Senecio*. It is assumed that he was examining Bentham's type material. Furthermore, Gibson (1969: 100) states that he saw a drawing and fragment of *A. S. Örsted 192* (GH) and that it was made from K of the lectotype.

Roldana heterogama is a common Central American species and has eccentrically peltate leaves. The variety *kellermanii* was based on plants having larger leaf blades, longer petioles, and the involucre villous-hirsute with red or reddish brown hairs. These characters are not inconsistent with the species as a whole; therefore, the variety is placed in synonymy. Upon examining the protologue and type material for *Cacalia calotricha*, it is also placed in synonymy.

Representative specimens examined. COSTA RICA. **Cartago:** ca. 19–20 km NW of Cerro Asunción (Cerro de la

Muerte) on C.R. #2, 19.6 km beyond El Empalme, 2500 m, 23 Dec. 1973, *Almeda 1091* (F). **Puntarenas:** Cantón de Perez Zeledon, Cordillera de Talamanca, 9°32′28″N, 83°35′04″W, 2600–2800 m, *R. Aquilar 3951* (MO). **San José:** along the trail from Canaan to Chirripó via Los Angeles, N (above) the Río Talari, 3100 m, 19–22 Jan. 1970, *Burger 7398* (NY). GUATEMALA. **Chimaltenango:** slopes of Volcán de Acatenango, above Las Calderas, 2800 m, 3 Jan. 1939, *P. C. Standley 61895* (F). **Guatemala:** Volcán de Pacaya, above Las Calderas, 2100 m, 30 Nov. 1938, *P. C. Standley 58356* (F). **Huehuetenango:** rd. to Huehuetenango, 7 mi. S of San Juan Ixcoy, 3000 m, 4 Feb. 1965, *D. E. Breedlove 8475* (F, MICH); Cerro Pixpix, above San Ildefonso Ixtahuacan, 2200 m, 15 Aug. 1942, *J. A. Steyermark 50556* (F, MO). **Quezaltenango:** Fuentes Georginas, W slope of Volcán de Zunil, 2850 m, 4 Mar. 1939, *P. C. Standley 67453* (F, MO); SE of Palestina, on old rd. to San Juan Ostuncalco, 2650 m, 21 Jan. 1941, *P. C. Standley 84269* (F). **Quiché:** Valle de Quiche, 2200 m, Apr. 1892, *Smith 3390* (F, GH, MO, NY). **Sacatepéquez:** slopes of Volcán de Agua, above Santa María de Jesús, 2500 m, 11 Feb. 1939, *P. C. Standley 65085* (F). **San Marcos:** betw. San Sebastian & summit of Volcán Tajumulco, 4200 m, 13 Feb. 1940, *J. A. Steyermark 35567* (F, MO); rd. betw. San Sebastian at Km 21 & Km 8, 8–18 mi. NW of San Marcos, 3200 m, 15 Feb. 1940, *J. A. Steyermark 35616* (F). **Sololá:** Volcán Tolimán (side facing Volcán Atitlán to summit), 2900 m, 13 June 1942, *J. A. Steyermark 47582* (F, MO). **Totonicapán:** on steep slopes of María Tecun, 3300 m, 12–23 Jan. 1966, *A. Molina R. 16400* (NY). MEXICO. **Chiapas:** on the SE side of the summit of Volcán Tacaná, mpio. of Union Juarez, 3600 m, 3 Mar. 1972, *D. E. Breedlove 24295* (MO); SW of Mexican hwy. 190 near Rancho Nuevo, ca. 9 mi. SE of San Cristóbal de las Casas, mpio. San Cristóbal de las Casas, 3000 m, 5 Mar. 1965, *D. E. Breedlove 9227* (DS, MICH, NY). **Oaxaca:** along hwy. 175 betw. Oaxaca & Pochutla, 7.3 mi. N of Suchixtepec, 2750 m, 21 Jan. 1979, *T. B. Croat 46166* (MO). PANAMA. **Bocas del Toro:** Cordillera de Talamanca, headwaters of the Río Culubre, 6 airline km NW of the peak of Cerro Echandion on the Costa Rican–Panamanian international border, 9°5′N, 82°50′W, 2500 m, 2 Mar. 1984, *Davidse 25154* (MO). **Chiriquí:** just below summit, El Baru, 3000 m, 20 Mar. 1977, *D'Arcy 11028* (MO).

22. Roldana heteroidea (Klatt) H. Rob. & Brettell, Phytologia 27: 420. 1974. *Senecio heteroideus* Klatt, Leopoldina 24: 125. 1888. *Digitacalia heteroidea* (Klatt) Pippen, Contr. U.S. Natl. Herb. 34: 380. 1968. TYPE: Mexico. Oaxaca: inter S. Andres et S. Maiguel, *F. M. Liebmann 178* (holotype, C not seen; isotype, GH not seen, C drawing GH!, C tracing MO!).

Cacalia longipetiolata B. L. Rob. & Greenm., Amer. J. Sci. Arts, ser. 3, 50: 157. 1895. TYPE: Mexico. Oaxaca: on dry ledges of the Sierra de San Felipe, 7000 ft., 10 Oct. 1894, *C. G. Pringle 5828* (holotype, GH not seen; isotype, US not seen).

Large perennial herbs, 1–2 m tall; plants essentially glabrous; stems terete, striate, cream to red. *Leaves* cauline, clustered at the midstem; petioles 8–12 cm, glabrous; blades 7–10 × 9–13 cm, palmately nerved, palmatifid, 5-lobed, sinus depth about 3/4 of the way to the midrib, lobes acute, secondarily lobed to merely denticulate; abaxially glabrate with short glandular hairs along veins and margins, adaxially glabrous. *Capitulescence* simple paniculate cyme, 2 to 5 capitula per branch, peduncles glabrous, primary bracts oblanceolate, 1.5–2.5 mm, ultimate peduncles 35–130 mm, bracteoles 0 to 3, linear, ca. 4 mm. *Capitula* eradiate (? or radiate), 12–18 × 5–8 mm, campanulate, corollas pale yellow; calyculate bracts ca. 3, linear, 5–7 mm; phyllaries ca. 8, glabrous, greenish purple-tinted, 12–15 × ca. 4 mm. Disk florets 30 to 40, corolla funnelform, glabrous, ca. 10 mm, tube ca. 7 mm, throat ca. 1 mm, lobes 2–3 mm. Achenes glabrous, cylindrical, ca. 2 mm, 10 ribs, resin glands absent; pappus bristles 7–8 mm.

Distribution and phenology. Mexico (Oaxaca) only at elevations of 2400–2700 m. Found in pine-oak forests. Collected in flower October through December.

Comments. *Roldana heteroidea* is a unique species with a very restricted distribution. It has only been collected along the ledges of the Sierra de San Felipe in Oaxaca.

However, a collection by *T. F. Daniel 368* (MICH, ASU: Queretaro, along hwy. 120, betw. Jalpan and Cadereyta) could be a variant of *Roldana heteroidea*. Its distinctive features are the ca. 15-mm-long ray ligules and bright green deeply 5-lobed to dissected palmate leaves. Its taxonomic determination depends on further investigation.

Representative specimens examined. MEXICO. **Oaxaca:** Cerro de San Felipe, 7500 ft., 23 Sep. 1895, *C. Conzatti 715* (GH); dry talus of ledges, Sierra de San Felipe, 8500 ft., 2 Dec. 1895, *C. G. Pringle 6176* (F, GH, MO, NY); Cerro de San Felipe, 7500 ft., Oct. 1894, *C. L. Smith 287* (F, MO, NY).

23. Roldana hintonii H. Rob. & Brettell, Phytologia 27: 420. 1974. *Senecio hintonii* (H. Rob. & Brettell) Pruski & T. M. Barkley, Phytologia 67: 238. 1989. TYPE: Mexico. México: Temascáltepec, Comunidad, upper oak belt, 1 m high, 8 Mar. 1936, *G. B. Hinton 8967* (holotype, US not seen, US photos F!, GH!, MO!; isotypes, F!, GH!, MICH!, MO!, NY!, UC!).

Suffruticose herbs to subshrubs, 1–2.5 m tall, arising from a woody caudex; new growth appressed arachnoid pubescent; stems terete, light brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 1–4 cm, pubescent; blades 8–16 × 2.5–5 cm, pinnately veined, elliptic, apex acute, base cuneate, margins entire to subentire, minutely callous denticulate; surfaces glabrate, appressed arachnoid hairs on veins, may be densely so abaxially. *Capitulescence* pyramidal compound panic-

ulate cyme, 10 to 25 capitula per branch, peduncles pubescent with appressed arachnoid hairs, primary bracts linear, ca. 2 mm, ultimate peduncles 9–11 mm, bracteoles 0 to 4, scale-like, to 1 mm. *Capitula* radiate, 8–12 × 2–3 mm, funnelform, corollas yellow; calyculate bracts ca. 3, scale-like, to 1 mm; phyllaries, ca. 13, glabrous, greenish purple-tipped, 4–6 × 1–2 mm. Ray florets 6 to 8, corolla ligulate, glabrous, tube 3–5 mm, ligule 4–6 × ca. 2 mm. Disk florets ca. 12, corolla funnelform, glabrous, 7–10 mm, tube 2–4 mm, throat ca. 4 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles 6–8 mm.

Distribution and phenology. Central Mexico. Known only from the vicinity of Temascaltepec, Mexico, at elevations of 2100–3000 m. Found in pine-oak and fir forests. Flowering February through March.

Comments. *Roldana hintonii* is a rarely collected species due to its restricted distribution. At first glance, it may appear to be a misplaced *R. schaffneri*. However, *R. hintonii* has 13 phyllaries versus 5 or 6 in *R. schaffneri*, and it has more numerous and larger ray and disk florets.

Representative specimens examined. MEXICO. **México:** Las Cruces, mpio. Temascáltepec, 2900 m, 16 Feb. 1933, *G. B. Hinton 3284* (F, GH); Communidad, 1 Mar. 1936, *G. B. Hinton et al. 8949* (GH, MICH, NY, UC); Las Cruces, 8 Mar. 1936, *G. B. Hinton 8966* (DS, F, GH, MICH, MO, NY); 22 km NW of Temascáltepec on rd. to Toluca, 19°06′58″N, 99°56′24″W, 2160 m, 24 Mar. 1996, *T. M. Barkley et al. 4030* (KSC, MO).

24. Roldana hirsuticaulis (Greenm.) Funston, Novon 11: 304. 2001. *Senecio hirsuticaulis* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 280. 1907. TYPE: Mexico. San Luis Potosí: en route from San Luis Potosí to Tampico, Dec. 1878 to Feb. 1879, *Dr. E. Palmer 1114* (holotype, GH!; isotype, NY!).

Suffruticose herbs to shrubs, 2 m tall; new growth tawny, lanate tomentose; stems terete, reddish brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 5–6 cm, lanate tomentose; blades 11–14 × 9.5–13.5 cm, triple-nerved, ovate to cordate, 9(+)-lobed, sinus depth less than 1/8 of the way to the midrib or merely dentate, abaxially densely lanate tomentose and canescent, adaxially pubescent along veins with long thin hairs. *Capitulescence* flat-topped compound paniculate cyme, 20 to 40 capitula per branch, peduncle lanate tomentose, primary bracts linear, ca. 5 mm, ultimate peduncles 2–10 mm, bracteoles ca. 2, linear, 1–2 mm. *Capitula* radiate, ca. 8 × 2 mm, turbinate, corollas yellow; calyculate bracts ca. 3, linear, 1–2 mm; phyllaries ca. 13, glabrous, greenish purple-tinted, 3–5 × ca. 1 mm. Ray florets ca. 3, corolla inconspicuously ligulate, pubescent, tube ca. 4 mm, ligule ca. 1 × 1 mm. Disk florets ca. 7, corolla funnelform, pubescent, ca. 7 mm, tube ca. 3 mm, throat ca. 2 mm, lobes ca. 2 mm. Achenes glabrous, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles ca. 5 mm.

Distribution and phenology. Eastern Mexico (Nuevo León, San Luis Potosí, Tamaulipas) at elevations of 2000–2500 m. Found in pine-oak forests. Flowering December through February.

Comments. The differences between *Roldana hirsuticaulis* and *R. aschenborniana* are minor, and much consideration was given to placing *R. hirsuticaulis* in synonymy. The species is recognized due to the persistent tomentum and often tawny pubescence found on the stem, peduncles, and underside of the leaf. *Roldana aschenborniana* typically is less densely pubescent and never tawny. *Roldana hirsuticaulis* seems to be restricted in distribution to the northern extreme of *R. aschenborniana*'s range. Gibson (1969) believed the type material for *R. hirsuticaulis* to be a hybrid between *R. aschenborniana* and *R. lanicaulis*. The relationships between these species are in need of more detailed analysis.

Representative specimens examined. MEXICO. **Nuevo León:** trail from Potrero Redondo to Laguna Sanchez, mpio. Villa Santiago, 16 Aug. 1939, *Muller 2748* (GH, MICH, UC). **San Luis Potosí:** chiefly in the region of San Luis Potosí, 22°N latitude, 2000–2500 m, 1878, *Parry & Palmer 539* (GH); en route from San Luis Potosí to Tampico, Dec. 1878 to Feb. 1879, *E. Palmer 1114* (GH, NY). **Tamaulipas:** 5 mi. NW, vic. of Gomez Farias, *Harrell 359* (MO); W of Gomez Farias on rd. to Rancho del Cielo, *McCarten 2605* (MICH); N of Frank Harrison's, Rancho del Cielo, in Sierra de Guatemala above Gomez Farias, *Sharp 52267* (NY).

25. Roldana jurgensenii (Hemsl.) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Senecio jurgensenii* Hemsl., Biol. Cent.-Amer., Bot. 2: 242. 1881. TYPE: Mexico. Oaxaca: San Pedro Nolasco, NE by E from Oaxaca, Feb. 1845, *Jurgensen 309* (holotype, K not seen; isotypes, G not seen, K fragm. & drawing GH!, G photos F!, GH!, MICH!, MO!).

Roldana breedlovei H. Rob. & Brettell, Phytologia 27: 416. 1974. TYPE: Mexico. Chiapas: SW of Mexican Hwy. 190 near Rancho Nuevo, ca. 9 mi. SE of San Cristóbal de las Casas, steep slope near crest of ridge 9000 ft., 5 Mar. 1965, *D. E. Breedlove 9228* (holotype, US not seen, US photos F!, GH!, MO!; isotype, NY!).

Suffruticose herbs, shrublets, or shrubs, 1–4.5 m tall; new growth densely covered with a combination of short stipitate-glandular and long multicelled hairs,

glabrate with age; stems terete, maculate, color varying from green leaves with purple stems to reddish brown leaves and stems, numerous lenticels either present or completely absent. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 5–14.5 cm, glabrous; blades 10–15 × 8–14.5 cm, triple-nerved, oval-ovate to subpalmatifid, 5- to 7-lobed, sinus depth less than 1/4 of the way to the midrib, lobes acute, weakly secondarily lobed to merely denticulate; surfaces glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* pyramidal compound paniculate cyme, 50 to 70 capitula per branch, peduncles densely pubescent with stipitate-glandular and long multicelled hairs, primary bracts linear, 2–4 mm, ultimate peduncles ca. 6 mm long, bracteoles 0 to 3, linear to scale-like, 1–2 mm. *Capitula* radiate, 6–11 × 2–3 mm, turbinate, corollas yellow; calyculate bracts 0 to 3, linear, ca. 2 mm; phyllaries ca. 8, stipitate-glandular pubescent to glabrate, purple-tinted, ca. 5 × 1.2 mm. Ray florets ca. 5, corolla ligulate, glabrous, tube (0.5)6–7 mm, ligule (4.5)6–7 × (1)2–3 mm. Disk florets ca. 7 to 13, corolla funnelform, glabrous, 6–8 mm, tube (1–2)4–5 mm, throat ca. 3 mm, lobes ca. (1)2 mm. Achenes glabrous, cylindrical, 1–3 mm, ribs 10, resin glands absent; pappus bristles 5–6 mm.

Distribution and phenology. Southern Mexico through Central America into Honduras (El Salvador: Santa Ana; Guatemala: Chimaltenango, Huehuetenango, El Progreso, Quezaltenango, Quiché, San Marcos, Sololá, Suchitepéquez, Totonicapán; Honduras: Lempira, Santa Bárbara; Mexico: Chiapas, Oaxaca) at elevations of 1000–3000 m. Found in montane cloud forests, open meadows, mixed broadleaf and cypress forests, and moist hillside thickets. The leaves are sold in Totonicapán markets for wrapping tamales. Flowering December through February.

Comments. *Roldana jurgensenii* is one of the more common species in Central America. Robinson and Brettell (1974) established the species *R. breedlovei* on the basis that it has more truncate leaf bases, less closely palmate leaf veins, throats of the corollas much shorter, and stems smooth (vs. raised lenticels). From my examination of the available collections, I believe that the species described by Robinson and Brettell is at one end of a morphological continuum. In the southern distribution, the complex has small capitula, dark brown leaves, and numerous raised light-colored lenticels on the stems and leaf blades. In the north, the capitula are larger with longer ray ligules and leaf blades are light green abaxially, dark green adaxially, and without lenticels. These characters states were also found to overlap to such an extent that the designation of separate taxa was not tenable. With regard to leaf morphology, leaf shape and venation in the genus are notoriously variable, and it holds true in this species as well.

Representative specimens examined. EL SALVADOR. **Santa Ana:** edge of cloud forest near summit of Los Esesmiles, 14°21′N, 89°09′W, 2580 m, 10 Mar. 1942, *J. M. Tucker 987a* (F, UC). GUATEMALA. **Chimaltenango:** Cerro Chichoy near Chichoy, where dept. of Chimaltenango, Quiche & Sololá join, ca. 14°48′N, 91°W, 2800–3200 m, 26–27 Jan. 1949, *L. O. Williams 15324* (F); cypress forest, Cerro de Tecpam, region of Santa Elena, 2400–2700 m, 26 Dec. 1938, *P. C. Stanley 60995* (F, MO). **Huehuetenango:** rd. to Huehuetenango, 7 mi. S of San Juan Ixcoy, mpio. San Juan Ixcoy, Sierra de los Cuchumatanes, 9750 m, 4 Feb. 1965, *D. E. Breedlove 8514* (DS, F, MICH); Sierra Cuchumatanes betw. Paquix & San Juan Ixcoy, 3000–3350 m, 8 Jan. 1974, *A. Molina R. 30071* (F, MO, NY). **El Progreso:** uppermost portion of slopes, betw. Calera & summit of Volcán Siglo, 2000–3300 m, 21 Jan. 1942, *J. A. Steyermark 43100* (F, GH, MO, NY). **Quezaltenango:** Canton La Esperanza, ca. 6 km from San Juan Ostuncalco, 3200 m, 12–23 Jan. 1966, *A. Molina R. 16596* (F, MO). **Quiché:** Chiul, 8000 ft., Apr. 1892, *Heyde et Lux. 2276* (F, GH). **San Marcos:** wet mtn. forest near Aldea Fraternidad, betw. San Rafael Pie de la Cuesta & Palo Gordo, W-facing slope of the Sierra Madre Mtns., 1800–2400 m, 10–18 Dec. 1963, *L. O. Williams et al. 25818* (F). **Sololá:** Volcán Santa Clara, S-facing slopes to summit, 2100–3000 m, 5 June 1942, *J. A. Steyermark 46973* (F). **Suchitepéquez:** Volcán Atitlán, S side, 7800 ft., 14 Jan. 1935, *A. F. Skutch 2130* (A, F, MO, NY). **Totonicapán:** region of Chiu Jolom, mtns. above Totonicapán, on rd. to Desconsuelo, 2800–3100 m, 23 Jan. 1941, *P. C. Standley 84415* (F, MO). HONDURAS. **Lempira:** Siquatepeque, Campo Narangjo, 14°33′N, 88°40′W, 2455 m, 30 Jan. 1992, *P. House 1183* (MO). **Santa Bárbara:** upper rocky slopes & summit of Cerro Santa Barbara, 2750 m, 5–6 Apr. 1951, *R. Armour 6089* (F). MEXICO. **Chiapas:** on the NE slope of Zontehuitz near the summit, mpio. Chamula, 9300 ft., 2 Dec. 1964, *D. E. Breedlove 7801* (D, MICH, MO); rd. from Motozintla de Mendoza to Siltepec via El Porvenir, 14.1 mi. NW of Motozintla, cloud forest on steep slopes facing the Atlantic, principal forest, 11 Feb. 1979, *T. B. Croat 47298* (KSC, MO). **Oaxaca:** 65.2 mi. N of the jct. of hwys. 175 & 190, 17 Dec. 1977, *V. Funk 2731* (OS).

26. Roldana kerberi (Greenm.) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Senecio kerberi* Greenm., Ann. Missouri. Bot. Gard. 1: 279. 1914. TYPE: Mexico. Colima: Mesa del Arrero, "trompetero," 21 Nov. 1880, *E. Kerber 94* (lectotype, designated here, B fragm. & tracing at GH!, B photos F!, GH!, MICH!, MO!, NY!, B tracing MO!).

Senecio galicianus McVaugh, Contr. Univ. Michigan Herb. 9: 473. 1972. *Roldana galiciana* (McVaugh) H. Rob. & Brettell, Phytologia 27: 418. 1974. TYPE: Mexico. Jalisco: Sierra de Cacoma, ca. 40 km W of Ayutla, precipitous mountainsides, 3–5 mi. NW of San Miguel de la Sierra, 2000 m, 3 Nov. 1962, *R. McVaugh 22017* (holotype, MICH!; isotype, NY!).

Suffruticose herbs, 1.5–4.7 m tall; new growth lightly to densely arachnoid floccose or tomentose to rarely tawny; stems terete, striate, maculate, reddish brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles (2–)10–20 cm, glabrate with arachnoid hairs at axils; blades ca. (5–)10–35 × (2–)10–25 cm, palmate- to triple-nerved, leaf shape various with 7 to 13 lobes: oblong-ovate to oval, fan-shaped with distal lobe sinus depth ca. 1/2 of the way to the midrib, apex acute to rounded, proximal lobes sinus depth ca. 1/4 of the way to the midrib, lobes acute; or broadly ovate-elliptic, sinus depth ca. 1/4 of the way to the midrib throughout, lobes broadly to narrowly acute; margins weakly denticulate, weakly secondarily lobed to variously serrate; abaxially glabrate, sparsely to densely arachnoid pubescent to having a tomentum, adaxially glabrous to slightly pubescent on veins and margins. *Capitulescence* pyramidal or flat-topped compound paniculate cyme, 25 to 100 capitula per branch, peduncles glabrate to arachnoid floccose, primary bracts linear, 2–10 mm, ultimate peduncles 2–10 mm, bracteoles 0 to 3, linear to weakly filiform, ca. 1 mm. *Capitula* radiate, 5–9 × 2–4 mm, turbinate, corollas yellow to golden; calyculate bracts ca. 3, linear, ca. 2 mm; phyllaries 8 to 13, glabrous, 4–6 × 1–2 mm. Ray florets 3 to 6, corolla ligulate, glabrous or pubescent, tube 2–3 mm, ligule 3–6 × ca. 2 mm. Disk florets 5 to 11, corolla funnelform, glabrous or pubescent, 5–7 mm, tube 2–3 mm, throat ca. 2 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, 1–2 mm, ribs 10, resin glands absent; pappus bristles 3–6 mm.

Distribution and phenology. Mexico (Colima, Jalisco, Oaxaca) at elevations of 1500–2600 m. Found in pine-oak and fir forests. Flowering October through March.

Comments. The lectotype designation for *Senecio kerberi* was made according to the *International Code of Botanical Nomenclature* (Greuter et al., 2000: Art. 8.3, Ex. 5). In the protologue for *S. kerberi*, Greenman (1914) listed the type material as being a specimen in B and a tracing and fragments in GH. The Berlin material was destroyed in 1943. Greenman (1914: 279) states that he was permitted to make tracings and take fragments for the Gray Herbarium from the specimens he was studying at the Royal Botanical Museum of Berlin. It is apparent that the GH tracing done by Greenman is of the B specimen that is depicted in all of the photographs, as well as the MO tracing. Therefore, the GH fragment and tracing is determined to be of the type and, thus, eligible to be the lectotype.

Roldana kerberi superficially resembles *R. hartwegii*; however, it is a stouter plant with typically larger leaves. Greenman (1914: 280) notes in the protologue that it is "related to *Senecio hartwegi* Benth. and *S. reglensis* Greenm., but from these and from other species of sect. *Palmatinervii* to which it belongs it is readily distinguished by the somewhat elongate more or less fan-shaped and bluntly lobed leaves." It has been surmised (Gibson, 1969; Robinson & Brettell, 1974; McVaugh, 1984; Kowal, 1991; Turner, 2005) that the type is a specimen of the more recently circumscribed *S. galicianus* McVaugh (1972). He described *S. galicianus*, citing specimens that had been annotated by Gibson as "*S. roldana* var. *calvescens*," a variety never published, commenting that *S. galicianus* has few of the characteristics associated with *S. roldana* (*R. lobata*). In *Flora Novo-Galiciana* (1984: 829), McVaugh also states that he believes *S. galicianus* is of closer affinity to *S. roldana* (*R. lobata*) than to *S. hartwegii*. While this may be true, I do not completely dismiss an affinity to *R. hartwegii*.

For 77 years, the only recognized gathering of *Roldana kerberi* was the type material. In 1991, two gatherings were collected from the general area of the type: *R. R. Kowal & M. A. Wetter 3025*, as reported by Kowal (1991: 131) and *A. C. Sanders 10314* (MO!). Kowal also discusses the relationships between *R. kerberi*, *Senecio galicianus*, and *R. lobata*. He found that while *R. kerberi* is ecologically similar in habit with *R. lobata*, it is morphologically more similar to *S. galicianus*, differing from the latter in leaf morphology and pubescence type, the leaf blade being much more leathery with a tomentum formed by rough, septate hairs and having a rust-colored pubescence. Kowal provisionally accepted *R. kerberi* as distinct from *S. galicianus*. While I had originally intended to do the same in this manuscript, upon finding the Sanders gathering, *S. galicianus* is relegated to synonymy. McVaugh (1972) understood this possibility as he stated in the protologue: "It is possible *S. galicianus* is a synonym of *S. kerberi* Greenm."

The three species *Roldana hartwegii*, *R. kerberi*, and *R. lobata* variously overlap along the Sierra de Manantlán, Sierra Madre del Sur, and Sierra de Miahuatlán. The varieties recognized here are illustrations of the possible ecomorphologic variation and hybridization that may occur among any of the species of *Roldana* that have a broad distribution. The interested reader is directed to the *R. hartwegii* section of this paper, as well as Greenman (1914), Kowal (1991), McVaugh (1972), and Turner (1996, 2005) for further discussion on the interrelationships of these species and their habitats.

KEY TO THE VARIETIES OF *ROLDANA KERBERI*

1a. Leaf blade oblong-ovate to oval, distal lobes elongate, apex acute to rounded, sinus depth ca.

1/2 of the way to the midrib, proximal lobes acute with sinus depth ca. 1/4 of the way to the midrib; phyllaries 10 to 13; corollas glabrous . . . . . .26a. *R. kerberi* var. *kerberi*

1b. Leaf blade broadly ovate-elliptic, lobes variously acute, sinus depth ca. 1/4 of the way to the midrib; phyllaries 8; corollas pubescent.

2a. Leaf lobes broadly acute; capitulescence over-topped by upper foliage; plants arachnoid to densely tomentose, from Sierra Madre del Sur . . 26b. *R. kerberi* var. *calzadana*

2b. Leaf lobes narrowly acute; capitulescence not over-topped by upper foliage; plants glabrate-arachnoid to septate-tomentose, from Sierra de Manantlán . . . . . .26c. *R. kerberi* var. *manantlanensis*

26a. Roldana kerberi var. kerberi

Distinguishing characters. Suffruticose herbs, 1.5–4 m tall; new growth weakly arachnoid floccose. *Leaf* petioles (2–)10–15 cm, glabrate with arachnoid hairs at axils; blades ca. (5–)10–15 × (2–)10–17 cm, oblong-ovate to oval, distal lobes sinus depth 1/2 of the way to the midrib, apex acute to rounded, proximal lobes acute; abaxially glabrate to sparsely arachnoid pubescent, adaxially glabrate. *Capitulescence* pyramidal compound paniculate cyme, ultimate peduncles glabrate to arachnoid floccose. *Capitula* radiate, 6–9 × 2–4 mm; phyllaries 10 to 13. Ray florets 3 to 6, glabrous. Disk florets 6 to 11, glabrous, 5–7 mm.

Distribution. Found on Pacific slopes at the western end of the Trans-Mexican Volcanic Belt in the Sierra de Manantlán at elevations of 1200–2200 m.

Comments. The type specimen of *Roldana kerberi* consists of an inflorescence and what appears to be a foliaceous bract as opposed to a cauline leaf. Nevertheless, cauline leaves have traditionally been described as 5–10 × 2–8 cm and subarachnoid-tomentose abaxially. It is suggested here that the relatively smaller leaf size is an artifact of the type specimen and should not be taken as a strict descriptive characteristic. Regarding plant pubescence, as is often found in the genus, plants growing along the Pacific slopes of Mexico have a tendency toward persistent and denser pubescence in the southern ranges, while in the northern ranges new growth may be densely pubescent but has a greater tendency to become glabrate with age.

Representative specimens examined. MEXICO. **Colima:** Rancho El Jabali, 20 km (air) NNW of Colima in the SW foothills of Volcán de Colima, 19 26.3′N, 103 42.5′W, 1275 m, 6 Jan. 1991, *A. C. Sanders 10314* (MO). **Jalisco:** NW flank of the Nevada de Colima, ca. 23 rd. mi. W of Ciudad Guzman, 2000 m, 31 Dec. 1962, *A. Cronquist 9767* (GH, KSC, MICH, MO, NY); 12–15 mi. SSE of Autlán, on lumber rd. to Corralitos, 4–10 mi. above (SE of) Ahuacapan, 1500 m, 22 Nov. 1989, *R. McVaugh & Koetz 978* (MICH); precipitous mtns. ca. 40 km W of Ayutla, 3–6 mi. SE of El Carmen, 2100 m, 29 Nov. 1960, *R. McVaugh 21560* (MICH).

26b. Roldana kerberi var. calzadana

(B. L. Turner) Funston, comb. nov. Basionym: *Roldana calzadana* B. L. Turner, Phytologia 80: 276. 1996. TYPE: Mexico. Oaxaca: mpio. San Martin Peras, carretera Coicoyan de las Flores, Santiago Juxtlahuaca, 17 17′N, 98°11′W, "200 m de la desviacion a San Martin Peras," ca. 2535 m, 16 Feb. 1995, *J. I. Calzada 19738* (holotype, TEX not seen, TEX digital image TEX!; isotype, MEXU not seen).

Distinguishing characters. Suffruticose herbs, ca. 2 m tall; new growth tawny-puberulent. *Leaf* petioles 16–20 cm long, blades ca. 30–35 × 16–20 cm, broadly ovate-elliptic, sinus depth about 1/4 of the way to the midrib, lobes broadly acute; abaxially glabrate to arachnoid-tomentose, adaxially glabrate with hairs along major veins. *Capitulescence* flat-topped congested cymes, arachnoid-tomentose. *Capitula* radiate, 5–6 mm high; phyllaries 8. Ray florets ca. 3, tubes puberulent, ligule 4–6 × ca. 2 mm. Disk florets ca. 5, pubescent, 7–8 mm. (Description compiled from the literature.)

Distribution. Southern Mexico (Oaxaca) along the Sierra Madre del Sur at elevations of 2500–2600 m.

Comments. Turner (1996) separates *Roldana calzadana* from *R. manantlanensis* by its having larger leaves with more broadly acute (broadly deltoid) marginal lobes, each irregularly serrate, and having a tomentose versus arachnoid pubescence. Turner also suggests a difference in capitulescence: congested flat-topped cymes as opposed to pyramidal cymes. While the somewhat disjunct distribution between gatherings and the possible difference in capitulescence form is not enough for me to recognize the species, it is being given varietal rank. The difference in distribution is not great, and Turner (2005: 230) discusses that the area is also ecologically hospitable to *R. lobata*; therefore, as discussed above, it would not be at all surprising to find these two species growing together.

26c. Roldana kerberi var. manantlanensis

(R. R. Kowal) Funston, comb. nov. Basionym: *Senecio galicianus* var. *manantlanensis* R. R. Kowal, Brittonia 43: 109. 1991. *Roldana manantlanensis* (R. R. Kowal) B. L. Turner, Phytologia 80: 278. 1996. TYPE: Mexico. Jalisco: 23 km SSE of Autlán, 17 km SSW of El Chante, on or below NW-SE crest of Sierra de Manantlán Occidental, on lumber rd. from Cerro La Cumbre 4 km NW toward las Joayas, ca. 1 km NE of

Cerro El Almeal, at spring above rd. and along brook below, 19°33–34′N, 104°14.5–15.5′W, 2090 m, 2 Jan. 1980, *R. R. Kowal 2776* (holotype, WIS!; isotype, MICH!).

Distinguishing characters. Suffruticose herbs, 2–4.7 m tall; new growth weakly arachnoid floccose. *Leaf* petioles 16–20 cm, blades 30–35 × 16–25 cm, broadly ovate-elliptic, sinus depth about 1/4 of the way to the midrib, lobes acute to narrowly so; abaxially glabrate to arachnoid-tomentose, adaxially glabrate with hairs along major veins. *Capitulescence* flat-topped congested cymes, arachnoid tomentose. *Capitula* radiate, 5–6 mm high; phyllaries 8. Ray florets ca. 3, tubes puberulent, ligule 4–6 × ca. 2 mm. Disk florets ca. 5, pubescent, 7–8 mm.

Distribution. Found along the Sierra de Manantlán Occidental of Mexico (Colima, Jalisco). Elevation ca. 2000 m.

Comments. Kowal (1991) used discriminant analysis to support recognizing variety *manantlanensis*. The characters distinguishing it are acute (deltoid) lobes of the leaf blade, broader phyllaries, fewer phyllaries and florets in the capitula, and larger ray and disk florets. His analysis is based on only seven and four collections of varieties *galicianus* and *manantlanensis*, respectively, so more data are needed to more firmly substantiate the recognition of this variety.

Representative specimens examined. MEXICO. **Jalisco:** mpio. Cuautitlán, en el rancho Lamial carretera para rancho de la Joya, pine forests, ca. 2020 m, 10 Dec. 1982, *J. I. Calzda & Nieves H. 9461* (ZEA); in saddle betw. Cerro la Cumbre and Cerro El Almeal, called Cloud Camp, 19°33′40″N, 104°14′40″W, 2150 m, 12 Oct. 1982, *H. H. Iltis 28869* (KSC, MICH, WIS).

27. Roldana langlassei (Greenm.) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Senecio langlassei* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 283. 1907. TYPE: Mexico. "Michoacán et Guerrero: Sierra Madre, arbrissecu de 3–4 m, fleurs jaunes, sol granitique, 1600 m," 21 Sep. 1899, *E. Langlassé 1005* (lectotype, designated here, GH!; isotype, US not seen).

Suffruticose herbs to shrubs, ca. 3 m tall; new growth pubescent with glandular hairs; stems terete, maculate, greenish yellow. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 11.5–26 cm, sparsely pubescent; blades 12.5–25 × 12.5–35 cm, triple-nerved, ovate-orbicular, 11- to 13-lobed, sinus depth about 1/4 of the way to the midrib, lobes acute, slightly secondarily lobed; abaxially glabrate to pubescent with stipitate-glandular hairs, persistent on veins, adaxially glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* pyramidal compound paniculate cyme, 50 to 80 capitula per branch, peduncles densely pubescent with stipitate-glandular hairs, primary bracts linear, up to 5 mm, ultimate peduncles ca. 10 mm, bracteoles 0 to 4, linear, ca. 2 mm. *Capitula* radiate, 12–14 × 2–3 mm, funnelform, corollas yellow; calyculate bracts 1 to 3, linear, 1 mm; phyllaries ca. 10, pubescent with stipitate-glandular hairs, mildly purple-tinted, ca. 6 × 1 mm. Ray florets ca. 5, corolla ligulate, glabrous, tube ca. 5 mm, ligule ca. 7 × 2 mm. Disk florets ca. 12, corolla funnelform, glabrous, ca. 10 mm, tube ca. 5 mm, throat ca. 4 mm, lobes ca. 1 mm. Achenes glabrous, cylindrical, 1–2 mm, ribs 10, resin glands absent; pappus bristles ca. 8 mm.

Distribution and phenology. Mexico (Guerrero) at elevations of 1600–2700 m. Pacific slopes of pine-oak forests with *Pinus*, *Abies*, *Clethra*, and *Quercus*. Flowering December through February.

Comments. Greenman (1907) cited *Langlassé 1005* (B, GH) in the protologue; the Berlin specimen was destroyed in 1943.

Roldana langlassei is an elusive and poorly collected species. Morphologically, it seems closer to the southern *R. petasitis* than with its geographically closer relatives.

Representative specimens examined. MEXICO. **Guerrero:** ca. 2 km al NE del Campamento El Gallo, estribacíones suroccidentales del Cerro Teotepec, ca. 17°28′N, 100°13′W, 2660 m, 27–29 Jan. 1965, *R. McVaugh 132* (DS, MICH); 27 km al NE del Paraiso, camino a Puerto del Gallo, mpio. Atoyac de Alvarez, 1720 m, 29 Mar. 1983, *Marinez S. 3804* (KSC).

28. Roldana lanicaulis (Greenm.) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Senecio lanicaulis* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 283. 1907. TYPE: Mexico. Chiapas: near Pinabete, 1800–2400 m, 8 Feb. 1896, *E. W. Nelson 3771* (lectotype, designated here, GH!; isotypes, MO!, US not seen).

Simple or sparingly branched herbs or subshrubs, 1–3 m tall; new growth densely white lanate tomentose, often tawny; stems terete, light brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 7–12 cm, tomentose; blades 15–25 × 12–22 cm, palmately nerved, reniform, 9(+)-lobed, sinus depth less than 1/8 of the way to the midrib, lobes acute, margins densely denticulate, occasionally denticles up to 3 mm long; abaxially densely lanate tomentose and canescent, adaxially sparsely pubescent with glandular hairs. *Capitulescence* round topped to pyramidal compound

paniculate cyme, 40 to 60 capitula per branch, peduncles floccose tomentose, primary bracts linear, up to 2 mm, ultimate peduncles 0–3(–5) mm, bracteoles 0 to 2, linear, to 1 mm. *Capitula* radiate, 5–8 × 2–3 mm, turbinate, corollas yellow; calyculate bracts ca. 3, linear or filiform, 1–3 mm; phyllaries 7 to 13, glabrous, purple-tinted, 3.5–5 × 1–2 mm. Ray florets 5 to 8, corolla ligulate, glabrous, tube 3–4 mm, ligule 2–4 × 1–2 mm. Disk florets 7 to 13, corolla funnelform to moderately campanulate, glabrous, 4–7 mm, tube 2–3 mm, throat 1–2.5 mm, lobes 1–1.5 mm. Achenes glabrous, cylindrical, 0.5–1 mm, ribs 5 or 10, resin glands absent; pappus bristles 4–7 mm.

Distribution and phenology. Restricted to the Sierra Madre Oriental and southward into Guatemala (Guatemala: Huehuetenango; Mexico: Chiapas, Oaxaca, Veracruz) at elevations of 1000–2500 m. Montane cloud forests with *Quercus*, *Pinus*, *Abies*, *Acer* L., *Styrax* L., and *Sauravia* Spreng. Flowering November through March.

Comments. In the protologue for *Senecio lanicaulis*, Greenman (1907) cited two syntypes: Guatemala. Dept. Quiché, Chiul, 2400 m, Apr. 1892, *Heyde & Lux 3377* (GH not seen); and *E. W. Nelson 3771* (GH, US).

Roldana lanicaulis is a distinct species from southern Mexico and Guatemala. It is distinguished by its tawny pubescence and reniform leaf blades that have numerous elongate denticles along the margins.

Representative specimens examined. GUATEMALA. **Huehuetenango:** wet cloud forest at Cruz de Limon, betw. San Mataeo Ixtatán & Nuca, Sierra de los Cuchumatanes, 2800 m, 31 July 1942, *J. A. Steyermark 49830* (F, MO [1256275, 1256274]). MEXICO. **Chiapas:** on ridge above Siltepec on the rd. to Huixtla, mpio. Siltepec, 2200 m, 1 Feb. 1982, *D. E. Breedlove 58235* (CAS); near Pinabete, 1800–2400 m, 8 Feb. 1896, *E. W. Nelson 3771* (GH, MO). **Oaxaca:** 4.3 km W of desviacíon a Zacatepec, 2400 m, 23 Apr. 1983, *Torres 2677* (MO, NY). **Veracruz:** pasando el Ingenio del Rosario rumbo a Buena Vista, 2400 m, 28 Dec. 1986, *Cházaro 4307* (WIS).

29. Roldana lineolata (DC.) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Senecio lineolatus* DC., Prodr. 6: 427. 1837 [1838]. TYPE: Mexico. [state unknown]: in Mexico Cordillera de Guchilapa, *J. L. Berlandier 1227* (lectotype, designated by McVaugh, 1984: 836, G-DC not seen, microfiche IDC 800. 1141 I 4!, G-DC photos F!, MICH!, MO!).

Senecio sinuatus Kunth, Nov. Gen. Sp. (folio ed.) 4: 141. 1818, nom. illeg., non *Senecio sinuatus* Gilib., 1798. *Roldana sinuata* B. L. Turner, Phytologia 87: 245. 2005 [2006], nom illeg. superfl. TYPE: Mexico. Guanajuato: betw. Guanajuato & Santa Rosa, *Humboldt & Bonpland s.n.* (holotype, P not seen, B photos F!, GH!, MICH!).

Single-stemmed perennial herbs or subshrubs, 1–3 m tall, from a tuberous thickened fibrous-rooted caudex; new growth weakly arachnoid floccose to tomentose; stems terete or slightly ribbed, maculate, green to dark red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous or clustered at midstem when herbaceous; petioles 1–4 cm, pubescent; blades 10–30 × 6–19 cm, pinnately veined and lobed, 7 to 11 lobes, sinus depth to 1/3 of the way to the midrib, lobes acute to rounded, may be weakly secondarily lobed, leaf base cuneate; abaxially densely pubescent to lanate tomentose and canescent, adaxially glabrous. *Capitulescence* ascending compound corymbiform cyme, 15 to 40 capitula per branch, peduncles pubescent to densely arachnoid floccose, primary bracts linear, up to 5 mm, ultimate peduncles 5–20 mm, bracteoles 0 to 3, linear, 1–2 mm. *Capitula* radiate, 10–17 × ca. 3 mm, funnelform, corollas yellow; calyculate bracts 2 to 3, filiform, 1–7 mm; phyllaries 7 to 8, glabrous, sometimes purple-tinted, 8–9 × ca. 1 mm. Ray florets ca. 5, corolla ligulate, glabrous, tube 4–6 mm, ligule 6–10 × ca. 2 mm. Disk florets 9 to 13, corolla funnelform, glabrous, ca. 10 mm, tube 3–4 mm, throat 4–5 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, 1–2 mm, ribs 5, resin glands absent; pappus bristles 6–7 mm.

Distribution and phenology. Central and southern Mexico (Distrito Federal, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, Veracruz) at elevations of 2000–3500 m. Found in pine-oak forests with *Pinus*, *Abies*, *Quercus*, *Prunus*, *Arbutus*, *Senecio*, *Phytolacca*, *Baccharis*, *Symphoricarpus*, and *Alnus*. Flowering October through February (March).

Comments. In the protologue for *Senecio lineolatus*, de Candolle (1837) listed two syntypes from the same locality, *J. L. Berlandier 1226* (G-DC, lectotype) and *J. L. Berlandier 1227* (G-DC not seen, microfiche IDC 800. 1141 I 5!). Regarding *S. sinuatus*, Greenman notes, in hand, on the B specimen "doubtless a fragment of the type." Unfortunately, the Berlin specimen itself was destroyed in 1943.

Roldana lineolata is a common species in Mexico. To the novice, it may be confused with *R. heracleifolia*. See the discussion section under that species for an outline of diagnostic characters.

Representative specimens examined. MEXICO. **Distrito Federal:** La Campana, 5 km NW of Ajusco, 3000 m, 20 Dec. 1964, *J. Rzedowski 19310* (DS, MICH, UMO). **Guanajuato:** mtn. slopes ca. 3 km E of Santa Rosa, 2700 m, 11 Nov. 1970, *R. McVaugh 24204* (MICH).

Guerrero: steep slope near summit of Cerro Teotepec, 3000 m, 12 Nov. 1973, *D. E. Breedlove 36082* (CAS, MICH, MO). **Hidalgo:** along hwy. 130 betw. Poza Rica & Mexico City, near reservoir, 23 km W of Huauchinango, 23 Oct. 1986, *T. M. Barkley 3917* (KSC, NY, WIS). **Jalisco:** plateau center 4–5 km NNE of Cerro San Miguel & 4 km S of Rincón de Manantlán, 19°34′N, 104°12′W, 2300 m, 10 Jan. 1980, *R. R. Kowal 2838* (MICH, WIS). **México:** Llano Grande hasta la cima, 3000 m, 20 Nov. 1965, *Boege 26* (CAS); ca. 2 km N of summit betw. Toluca & Mexico City, on the northern route, 3100 m, 20 Oct. 1970, *A. Cronquist 10824* (CAS, GH, MICH, NY); 19 km E of Texcoco, 1 km S of rd. Texcoco–Calpulalpan, mpio. Tepetlaoxtoc, 2800 m, 28 Sep. 1976, *García P. 116* (CAS, MO). **Michoacán:** Mt. Patamban, 3200 m, 29 Jan. 1903, *E. W. Nelson 6578* (GH, MO); rd. to Los Azufres, 19 km by rd. from Mex. hwy. 15, 6 km W of Ciudad Hidalgo, 19°48′N, 100°37′W, 3400 m, 19 July 1975, *Steingraeber 21* (WIS). **Morelos:** on hwy. 95 betw. Mexico City & Cuernavaca, ca. 1 km N of Tres Marías, 17 Oct. 1986, *T. M. Barkley 3901* (KSC, WIS); Tres Marías, 2900 m, 7 Nov. 1903, *C. G. Pringle 11569* (CAS, F, GH, MICH, NMC). **Oaxaca:** left to La Cumbre, 17 mi. from Oaxaca, lt. on lumber rd. to lumber camp, 9 mi. along crest San Felipe range, 2900 m, 17 Jan. 1965, *Carlson 4012* (F, MICH, NY). **Puebla:** above & S of hwy. Mex. 190 betw. Puebla & Mexico City, 4 km E of Río Frio recreation area & Mexico–Puebla state line, 2800 m, 7 Jan. 1981, *M. Nee 19592* (NY). **Querétaro:** Puerto de Agua Fria, ca. 10 km S of Pinal de Amoles, mpio. Pinal de Amoles, 2800 m, 5 Sep. 1985, *Fernandez 3065* (NY). **Veracruz:** 8 km E of Los Altos Veracruz & 12 km W of Ayahualulco, 19°24′N, 97°12′W, 2900 m, 7 Nov. 1981, *M. Nee 22869* (F, WIS).

30. Roldana lobata La Llave, Nov. Veg. Descr., Fasc. 2: 10. 1825. *Senecio roldana* DC., Prodr. 6: 431. 1837 [1838], non *Senecio lobatus* Pers., 1807; nec *Senecio lobatus* Sessé & Moc., 1894. TYPE: Mexico. [s. loc.], 1831, *Alaman s.n.* (neotype, designated here, G-DC not seen, microfiche IDC 800. 1142 I 3–4!, G-DC photos F!, MO!).

Senecio schumannianus S. Schauer, Linnaea 20: 698. 1847. TYPE: Mexico. Hidalgo: ca. Zimapán, *Aschenborn 691* (lectotype, designated here, B fragm. GH!).

Senecio rotundifolius Sessé & Moc., Pl. Nov. Hisp. 140. 1887 [1890], nom. illeg., non *Senecio rotundifolius* Stokes, 1812; nec *Senecio rotundifolius* Lapeyr., 1813; nor *Senecio rotundifolius* Hook. f., 1853. TYPE: Mexico. [state unknown]: Xochitlán, *Sessé & Mociño 2820* (lectotype, designated here, MA not seen, MA photo MICH 1764!).

Senecio jaliscanus S. Watson, Proc. Amer. Acad. Arts 26: 143. 1891, as "jaliscana" TYPE: Mexico. Jalisco: Chapala Mtns., near Guadalajara, 9 Dec. 1889, *C. G. Pringle 2931* (holotype, GH!).

Roldana juxtlahuacana B. L. Turner, Phytologia 37: 230. 2005 [2006]. TYPE: Mexico. Oaxaca: Mpio. Santiago Juxtlahauaca, 6–7 km from El Manzana along rd. to Infiernillo, 17°12′N, 98°04′W, pine-oak forests, locally abundant, 13 Feb. 1996, *J. I. Calzada 20776* (holotype, TEX not seen, TEX digital image TEX!).

Single-stemmed perennial herbs or unbranched shrubs, 1–4 m tall, arising from a woody caudex; glabrate below, upwards new growth arachnoid pubescent to floccose tomentose, at times canescent; stems terete, red with patches of cream-green. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 7–13 cm, glabrescent, arachnoid pubescent to floccose tomentose; blades 15–25 × 10–15(–20) cm, triple-nerved, blade shape various: oval-subpalmatifid, 5- to 7-lobed, sinus depth 1/4 of the way to the midrib, lobes acute, first set of proximal lobes longer and broader than others, giving blade a rhomboid aspect; or ovate, (3 to)5- to 7-lobed, sinus depth less than 1/8 of the way to the midrib, lobes acute; or occasionally rotund-oval, margins subentire; all margins sparsely callous denticulate, abaxially arachnoid pubescent to lightly tomentose and canescent, adaxially glabrous. *Capitulescence* elongate pyramidal compound paniculate cyme, 10 to 30 capitula per branch, peduncles arachnoid pubescent to densely floccose tomentose, primary bracts linear, 2–5 mm, ultimate peduncles (0–)5–10 mm, bracteoles 0 to 2, linear, 1–2 mm. *Capitula* eradiate or radiate, 7–13 × 3–5 mm, turbinate, corollas yellow to light orange; calyculate bracts ca. 5, linear or filiform, 2–3 mm; phyllaries 10 to 13(to 14), arachnoid pubescent to tomentose, purple-tinted, 3.5–6.5 × 1–2 mm. Ray florets absent or 3 to 6, corolla ligulate, pubescent, hairs typically restricted to the top of the tube, tube 2–5 mm, ligule (2.5–)4–8 × 2–3 mm. Disk florets (11 to)15 to 22, corolla funnelform to moderately campanulate, pubescence typically restricted to the top of the tube, (? rarely glabrous), 5–8.5 mm, tube 2–3.5 mm, throat 2–4.5 mm, lobes 0.5–2 mm. Achenes glabrous, cylindrical, 1–3 mm, ribs 5 or 10, resin glands absent; pappus bristles 5–8 mm.

Distribution and phenology. Central and southwestern Mexico, Guatemala (Guatemala: Jalapa; Mexico: Distrito Federal, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Oaxaca) at elevations of 1300–2700 m. Ravines, steep mountainsides, in tropical subdeciduous forest with *Quercus*, *Fraxinus*, *Prunus*, and *Pinus*. Flowering October through February.

Comments. The type for *Roldana lobata* came from an unknown locality in Mexico and grown in the Royal Botanical Garden, Mexico City. Specifically, La Llave (La Llave & Lexarza, 1825) reports in the protologue "Januario floret in horto mexicano." The La Llave collections are cited in *Taxonomic Literature*, 2nd edition (TL-2), as deposited at G, but the type collection of *R. lobata* was not found in G by Laurent Gautier (pers. comm., Sept. 2004). A type collection of this plant is thus presumed nonexistent. When de Candolle (1837) made the nomen novum *Senecio roldana*, he cited two gatherings in addition to

the replaced synonym, *R. lobata*. He cited the gatherings with their herbarium names, which were nomina nuda and, hence, not validly published. These invalid names are included here, as they provide an additional reference when viewing the collections. The gatherings cited by de Candolle are both labeled from Mexico without locality: *Cineraria angulata* Alaman, nom. nud., 1831, *Alaman s.n.* (G-DC not seen, microfiche IDC 800. 1142 I 3–4!; G-DC photos F!, MO!); and *Cineraria lobata* Mairet, nom. nud., 1833, *E. M. Mairet s.n.* (K not seen, microfiche IDC 800. 1142 I 2!). Therefore, because no original material is available for *R. lobata*, the specimen *Alaman s.n.* (G-DC) is chosen here as the neotype.

Regarding *Senecio schumannianus*, Schauer (1847) lists *Aschenborne 691* in the protologue but does not designate a herbarium. No information is provided on Sebastian Schauer in TL-2. Nevertheless, there is a fragment from Berlin at GH, which is believed to be of the type material; because the B specimen was destroyed in 1943, the GH fragment, while mislabeled "Aschenborn 641," is chosen here as the lectotype.

No collection was cited in the protologue (1887) for *Senecio rotundifolius* Sessé & Moc.; the lectotype was chosen from original Sessé and Mociño material at MA.

Roldana lobata is a distinct and common species. Its sometimes rhomboid-like leaf shape and dense floccose to tomentose arachnoid pubescence are unique.

McVaugh (1984: 832) notes that material from Nueva Galicia, Jalisco, is strictly discoid, a feature used to circumscribe *Senecio jaliscanus*. However, McVaugh agrees that this distinction is not enough to separate the two species. He also states that the radiate form is known from central Michoacán eastward. I can neither confirm nor deny this observation. Gibson (1969: 149) contemplated the variety *calvescens* based on its being more coarse and with stems more herbaceous, having somewhat smaller heads, and a lack of floccose tomentum. Specimens he cited with these characters have been placed in *Roldana kerberi* or *R. petasitis* var. *oaxacana*.

Roldana juxtlahuacana differs from the type of *R. lobata* by having less pubescence. While this is noteworthy, it does not separate it from my species concept of *R. lobata*. In addition, there are plants to the north with less pubescence as well (México: *García 206*). While Turner (2005) reports the type specimen to have glabrous corolla lobes, which would be a possible distinguishing character, the material I have seen that fits the description and distribution of *R. juxtlahuacana* has weakly pubescent corolla lobes. Therefore, the species is placed in synonymy here until further specimens are examined.

Representative specimens examined. GUATEMALA. **Jalapa:** mtn. rd. betw. Jalapa & Paraíso, 1400–1700 m, *P. C. Standley 77351* (MO). MEXICO. **Distrito Federal:** 2600 m, 11 Nov. 1903, *C. G. Pringle 11571* (CAS, GH, MICH). **Guerrero:** 11 mi. W of Chilpancingo, rd. to Omiltemi, 1800 m, 21 Oct. 1962, *R. McVaugh 21907* (MICH). **Guanajuato:** Cerro Grande, cerca de San Diego, mpio. Acambaro, 2500 m, 27 Dec. 1967, *J. Rzedowski 25381* (MICH). **Hidalgo:** ca. Zimapan, *Aschenborn 691* (fragm. & drawing at GH). **Jalisco:** N of Sierra de Manantlán, 13 km S of El Chante, 19°36′N, 104°13′W, 1500 m, 14 Jan. 1980, *R. R. Kowal 2853* (MICH, WIS). **México:** 1 km N of rd. that runs betw. Tramo–Tlalmanalco–Amecameca, 2300 m, 12 Nov. 1976, *García 206* (CAS, F, NY); dist. Temascáltepec, 1500 m, 4 Dec. 1932, *G. B. Hinton 2859* (GH, MO, NY). **Michoacán:** S-facing slopes of mtns. betw. Río del Saltó & La Polvilla, ca. 18 mi. E of Morelia, 2200 m, 9 Nov. 1961, *King 5050* (MICH, NY). **Morelos:** along free rd. from Mexico City to Cuernavaca, 3 mi. S of Tres Cumbres, 3000 m, 16 Jan. 1966, *D. E. Breedlove 14231* (CAS, MICH). **Oaxaca:** along hwy. 175 betw. Oaxaca & Pochutla, 7.3 mi. N of Suchixtepec, 2800 m, 21 Jan. 1979, *T. B. Croat 46166* (KSC, MO); distr. Putla, 18 km N of Putla de Guerrero, 27 Oct. 1980, *O. Tellez 3883* (MO 4051240).

31. Roldana metepeca (B. L. Turner) C. Jeffrey, Kew Bull. 47: 55. 1992, as "metepecus." *Senecio metepecus* B. L. Turner, Phytologia 66: 465. 1989. TYPE: Mexico. Hidalgo: mpio. de Tenango de Doria, 18 km NNE of Metepec, "sobre camino de terraceria que va de Metepec a Tenango de Doria," 2200 m, 31 Oct. 1977, *J. García P. 514* (holotype, TEX not seen, TEX digital image TEX!; isotypes, CHAPA not seen, KSC!).

Suffruticose herbs and subshrubs, 1–4 m tall, spreading from creeping rhizomes; new growth stipitate-glandular pubescent with purplish elongate crinkled trichomes; stems terete, purple. *Leaves* cauline, clustered at base and midstem; petioles 7.5–10.5 cm, stipitate-glandular pubescent; blades 4.5–12 × 9–15 cm, triple-nerved, subpalmatifid to ovate, 5 to 7 lobes, lobes acute, weakly secondarily lobed to denticulate; abaxially densely stipitate-glandular with long multicelled hairs along veins, adaxially sparsely pubescent with short glandular hairs. *Capitulescence* simple paniculate cyme, 2 to 5 capitula per branch, peduncles densely stipitate-glandular pubescent, primary bracts linear-elliptic, ca. 8 × 3 mm, ultimate peduncles 24–40 mm, bracteoles 0 to 2, linear to weakly obovate, 2–5 × 2 mm. *Capitula* eradiate, ca. 16 × 4 mm, turbinate, corollas yellow; calyculate bracts ca. 3, linear, 5 mm; phyllaries 8 to 13, densely stipitate-glandular pubescent, purple-tinted, ca. 12 × 3 mm. Disk florets 20 to 30, corolla narrow, glabrous, ca. 10 mm, tube ca. 4 mm, throat ca. 4.5 mm, lobes ca. 1.5 mm. Achenes glabrous, cylindrical, ca. 3 mm, ribs 5, resin glands absent; pappus bristles ca. 10 mm.

Distribution and phenology. Central Mexico (Hidalgo, Puebla, Oaxaca) at elevations of 2000–2200 m.

Found in pine-alder forests. Collected in flower August and October.

Comments. *Roldana metepeca* is a poorly collected species. It has eradiate capitula and a densely stipitate-glandular pubescence. While other species in the genus have red stems and may have light purple trichomes, the deep violet purple stems and trichomes in this species are unique.

Representative specimens examined. MEXICO. **Hidalgo:** 8–11 km SW of Tenango de Doria, 1830 m, 30 Oct. 1983, *D. E. Breedlove 59570* (CAS). **Oaxaca:** Maria Tiltepec, 10 km SW of Totontepec, 2360 m, 20 Jan. 1984, *R. Torres C. 4608* (MO). **Puebla:** El Infiernillo, Dec. 1954, *E. Lyonnet 541200067* (MO).

32. Roldana mexicana (McVaugh) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Senecio mexicanus* McVaugh, Contr. Univ. Michigan Herb. 9: 473. 1972. TYPE: Mexico. Michoacán: hills of Pátzcuaro, 11 Nov. 1890, *C. G. Pringle 3332* (holotype, MICH!; isotypes, F!, GH!, MICH!, MO!, NY!, UC!).

Single-stemmed perennial herbs, 1–2.5 m tall, arising from a rhizomatous caudex; plants essentially glabrous; stems terete, striated, cream to dark red in color. *Leaves* cauline, clustered at midstem, reduced upwards; petioles 7.5–28 cm, glabrous; blades 8–28 × 8–28 cm, triple-nerved, suborbicular to subovate, 7- to 13-lobed, sinus about 1/4 of the way to the midrib, lobes acute or rounded, weakly secondarily lobed to merely denticulate; surfaces glabrous, margin and proximal veins of larger leaves with short glandular hairs. *Capitulescence* flat-topped to rounded compound paniculate cyme (umbel-like), 10 to 20 capitula per branch, peduncles glabrous, primary bracts linear, up to 5 mm, ultimate peduncles 3–15 mm, bracteoles 3 to 5, scale-like to linear, 2–3 mm. *Capitula* eradiate, 8–15 × 3–5 mm, funnelform, corollas orange to orange-yellow; calyculate bracts ca. 5, scale-like, 1–2 mm; phyllaries ca. 8, glabrous, greenish purple-tinted, 7–13 × 1–2 mm. Disk florets 9 to 12, corolla funnelform, glabrous, 8–10 mm, tube 3–5 mm, throat ca. 3 mm, lobes ca. 2 mm. Achenes pubescent, cylindrical, 1–2 mm, ribs 10, resin glands absent; pappus bristles 7–9 mm.

Distribution and phenology. Central Mexico (Guerrero, Jalisco, México, Michoacán, Oaxaca) at elevations of 1500–2500 m. Found in pine-oak forests with *Pinus*, *Quercus*, and *Alnus*. Flowering October through December.

Comments. *Roldana mexicana* is a distinct species that resembles *R. suffulta*; however, they are easily separable by the fewer florets (9 to 12) and scale-like calyculate bracts found in *R. mexicana* versus the numerous florets (90+) and obovate calyculate bracts found in *R. suffulta*.

Representative specimens examined. MEXICO. **Guerrero:** 36 km NE of Pueblo El Gallo, rd. to Filo del Caballo on ridge ENE of Teotopec, 2200 m, 13 Nov. 1973, *D. E. Breedlove 36123* (CAS, MICH); along hwy. betw. Milpillas (on hwy. 95) & Atoyac de Alvavez, 3.7 mi. W of turn-off onto rd. Chichihualco, 2300 m, 14 Jan. 1979, *T. B. Croat 45612* (MO). **Jalisco:** Cerro de Santa Fé, puente Grande Zapotlánejo, 1600 m, 21 Oct. 1972, *Díaz L. 3571* (F, MICH, NY, OS). **México:** Nanchititla, dist. Temascáltepec, 26 Nov. 1935, *G. B. Hinton 8745* (GH, MICH, NY). **Michoacán:** Cerro Azul, vic. of Morelia, 2100 m, 15 Sep. 1909, *Arsene 2697* (GH, MO); Pátzcuaro, *Holway 3182* (GH); oak zone 5 mi. N of Pátzcuaro, 2900 m, 25 Oct. 1962, *R. McVaugh 21929* (MICH). **Oaxaca:** mpio. Coicoyán, Sierra Sur, Juxtlahuaca, Siki Nami al SW de Coicoyán, 17°16′N, 98°17′W, 2100 m, 5 Nov. 1988, *A. de Avila 471* (MO).

33. Roldana mezquitalana (B. L. Turner) Funston, Novon 11: 305. 2001. *Senecio mezquitalanus* B. L. Turner, Phytologia 71: 56. 1991. *Senecio gesneriifolius* B. L. Turner, Phytologia 62: 75. 1987, nom. illeg., non *Senecio gesnerifolius* Cuatrec., 1950. *Roldana gesneriifolia* C. Jeffrey, Kew Bull. 47: 54. 1992, nom. superfl. TYPE: Mexico. Durango: mpio. Mezquital, 26.5 km de La Guajolota por el camino a Platanitos, ca. 2610 m, 15 Mar. 1985, *M. González 1677* (holotype, TEX not seen, TEX digital image TEX!).

Senecio floresiorum B. L. Turner, Phytologia 74: 367. 1993. Syn. nov. *Roldana floresiorum* (B. L. Turner) B. L. Turner, Phytologia 87(3): 222. 2005 [2006]. TYPE: Mexico. Jalisco: mpio. Totatiche, Rancho Acaspulco, 8 km al SW de Temastian, ca. 2000 m, 28 June 1991, *A. Flores M. & M. Flores M. 2688* (holotype, TEX not seen, TEX digital image TEX!; isotype, IGE not seen).

Suffruticose herbs or shrubs, ca. 2 m tall; new growth densely white tomentose; stems terete, dark red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 2–2.5 cm; blades 10–15 × 3–4 cm, pinnately veined, elliptical to elliptic-obovate, apex acute, base cuneate, margins irregularly serrulate; surfaces glabrate, pubescent on veins. *Capitulescence* terminal somewhat rounded corymbiform cyme, ca. 18 capitula per branch, peduncles densely stipitate-glandular pubescent, primary bracts linear, ultimate peduncles (3–5)6 mm, bracteoles 2 to 3, linear, ca. 1 mm. *Capitula* radiate, 7–8 × ca. 7 mm, campanulate, corollas yellow; calyculate bracts 6 to 8, linear, 1–2 mm; phyllaries 8 or 11 to 13, glabrous, (4–5)7–8 mm high. Ray florets 5 to 6, corolla ligulate, glabrous, tube ca. 5 mm, ligule (2–4)6–7 × 2.5–3 mm. Disk florets ca. 18, corolla funnelform, glabrous, ca. 8 mm, tube ca. 3.5 mm, throat ca. 3.5 mm, lobes ca. 1 mm. Achenes

glabrous, cylindrical, ca. 2 mm, ribs 5, resin glands absent; pappus bristles 5–7 mm. (Description compiled from the literature.)

Distribution and phenology. Mexico (Durango, Jalisco) at elevations of 2000–2600 m. Found in pine-oak forests. Collected in flower in March.

Comments. *Roldana mezquitalana*, like *R. hintonii*, superficially looks like *R. schaffneri*, but the former two species have phyllary numbers of 8 or 11 to 13 and occur in west-central to northern Mexico, whereas *R. shaffneri* has 5 to 6 phyllaries and is prolific in southern Mexico and Central America. *Roldana mezquitalana* is separated from *R. hintonii* by a denser pubescence, serrulate leaf margins, geography, and, in minor ways, by characters related to the capitulescence and capitula. I have only seen an illustration of this species, and it is possible that it is conspecific with *R. hintonii*.

Roldana floresiorum is described as being identical to *R. mezquitalana* except for differences in capitulescence morphology and in having smaller capitula. In my overall concept of the genus, species based on such differences, when they occur within the same range, are not recognized; therefore, *R. floresiorum* is placed in synonymy and the description of *R. mezquitalana* is broadened to include plants with ultimate peduncles 3–5 mm; phyllaries 8, 4–5 mm high; and ligules 2–5 mm.

34. Roldana michoacana (B. L. Rob.) H. Rob. & Brettell, Phytologia 27: 421. 1974. *Cacalia michoacana* B. L. Rob., Proc. Amer. Acad. Arts 43: 46. 1907. *Pericalia michoacana* (B. L. Rob.) Rydb., Bull. Torrey Bot. Club 51: 377. 1924. *Senecio michoacanus* (B. L. Rob.) B. L. Turner & T. M. Barkley, Phytologia 67: 392. 1989. TYPE: Mexico. Michoacán: on a pine-covered crater cone, Uruapan, 5500 ft., 31 Oct. 1905, *C. G. Pringle 10117* (holotype, GH not seen, GH photo MICH!; isotypes, F!, NY!).

Cacalia trigonophylla S. F. Blake, J. Wash. Acad. Sci. 19: 280. 1929. Syn. nov. TYPE: Mexico. Jalisco: in dense growth beside stream on steep hill, trail to La Sabala Mines, San Sebastian, Sierra Madre Mtns., 1500 m, 10 Feb. 1929, *Ynes Mexia 1656* (holotype, US 1318107 not seen, US photo MICH!; isotypes, F!, MICH!, NY!).

Small single-stemmed perennial herbs, 0.5–1.5 m tall, arising from a bulbous caudex with woolly buds; plants essentially glabrous, may have scattered pubescence of short stipitate-glandular hairs; stems terete, striated, greenish to red in color. *Leaves* cauline, clustered at midstem; petioles 5–12 cm, glabrous; blades 4–9 × 4–13 cm, triple-nerved, subpalmatifid, 3- to 5(7)-lobed, sinus depth about 1/4 of the way to the midrib, lobes acute, weakly secondarily lobed to denticulate; surfaces glabrous, margins and proximal veins may be weakly pubescent on larger leaves. *Capitulescence* simple to compound paniculate cyme, 1 to 4 capitula per branch, peduncles glabrous, primary bracts linear, ca. 3 mm, ultimate peduncles 10–100 mm, bracteoles 0 to 3, linear, 2–5 mm. *Capitula* eradiate, 10–20 × 3–6 mm, campanulate, corollas yellow-cream or white; calyculate bracts 5 to 10, filiform, 2–10 mm; phyllaries ca. 13, glabrous, purple-tinted, 10–12 × 1–2 mm. Disk florets 36 to 58, corolla funnelform to slightly campanulate, glabrous, 10–13 mm, tube 4–5 mm, throat 4–6 mm, lobes 2–3 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 8–11 mm.

Distribution and phenology. Central Mexico (Jalisco, México, Michoacán) at elevations of 1500–2500 m. Pacific slopes, pine-oak and fir forests with *Pinus*, *Quercus*, *Ostrya*, *Cornus*, *Meliosma*, and *Podocarpus*. Flowering November through January.

Comments. *Roldana michoacana* is a somewhat smaller version of *R. suffulta*. While these two species are difficult to separate, at times they are distinct. *Roldana michoacana* has filiform calyculate bracts, glabrous achenes, a simple to compound paniculate cyme, and typically smaller capitula. *Roldana suffulta* has obovate calyculate bracts, pubescent achenes, a compound corymbiform cyme, and typically larger capitula.

Representative specimens examined. MEXICO. **Jalisco:** betw. Santa Monica & N to Aserradero Agua Blanca, ca. 20°N, 104°30′W, 2275 m, 13 Nov. 1952, *R. McVaugh 14136* (MICH, NY); steep mtns. 11–12 mi. S of Talpa de Allende, headwaters of W branch of Río de Talpa, 1450 m, 24 Nov. 1960, *Pippen 63* (MICH, WIS). **México:** 8 km NE of Temascaltpec, 2070 m, 25 Nov. 1983, *D. E. Breedlove 60485* (CAS). **Michoacán:** dist. Coalcomán, 1740 m, 5 Dec. 1938, *G. B. Hinton 12718* (GH, MICH, NY); 17 km W of Ciudad Hidalgo, carretera Morelia, por Mil Cumbres, mpio. Ciudad Hidalgo, 2450 m, 11 Oct. 1983, *Martínez S. 4717* (KSC).

35. Roldana mixtecana Panero & Villaseñor, Brittonia 48: 83. 1996. TYPE: Mexico. Oaxaca: Dist. Juxtlahuaca, Cañada del Río Ratón a lo largo de la nueva brecha que lleva a campos de fresas al W de Cañada Lobos, 1895 m, 17°19′53.7″, 98°08′12″W, 24 Oct. 1994, *J. L. Panero, E. Manrique & J. I. Calzada 5409* (holotype, MEXU not seen; isotypes, MSC not seen, TEX not seen, TEX digital image TEX!).

Perennial herbs, 0.5–1 m tall; plants sparsely to moderately pubescent, arachnoid, burgundy; stems 3 to 5, only 1 to 3 producing flowers, terete. *Leaves* cauline, evenly distributed along stem, reduced

upwards; petiole 0.3–2.7 cm; blade 1.3–6 × 1.3–6.5 cm, subcordate-deltoid, 5 shallow lobes to merely 5 or 3 dentate; surfaces glabrescent. *Capitulescence* simple corymbiform cyme, 3 to 24 capitula per capitulescence, peduncles 2.5–6 cm, sparsely pubescent, green. *Capitula* radiate, 1.2–1.5 × 0.7–1 cm, campanulate, corollas bright yellow; phyllaries 25 to 35 in 5 graduated somewhat dimorphic series; phyllaries of first to third series 2–7 × 1.2–1.7 mm, appressed, chartaceous, ovate to lanceolate, glabrous, stramineous-green rimmed with purple; phyllaries of fourth to fifth series ca. 10 × 1.7–2 mm, appressed, chartaceous, ovate to oblong, glabrous, stramineous. Ray florets 5 to 7, corolla ligulate, glabrous, tube ca. 4 mm, ligule 9–10 × 3–4 mm. Disk florets 20 to 27, corolla campanulate, glabrous, ca. 8 mm, tube ca. 3.5 mm, throat 2 mm, lobes 2–2.5 mm. Achenes glabrous, columnar, ca. 2.5 mm; pappus bristles 5.5–6.5 mm. (Description compiled from the literature.)

Distribution. Mexico (Oaxaca), elevation ca. 2000 m. Found on well-drained, shady, rocky slopes of pine-oak forests.

Comments. *Roldana mixtecana* is morphologically an outlier in the genus due to its multiseriate phyllaries, but in all other aspects it has the gestalt of *Roldana*. The authors compare it to *R. michoacana* and *R. sessilifolia* in the protologue. My initial impression was that it was the rarely collected *R. hederifolia*; however, the multiseriate phyllaries argue against this. Having seen only a digital image of the type specimen, I will refrain from further comment and recognize the species as circumscribed.

36. Roldana neogibsonii (B. L. Turner) B. L. Turner, Phytologia 80: 278. 1996. *Senecio neogibsonii* B. L. Turner, Brittonia 37: 119. 1985. *Roldana neogibsonii* (B. L. Turner) Funston, Novon 11: 304. 2001, isonym. TYPE: Mexico. Veracruz: mpio. Huayacocotla, orilla del camino entre Helechales y Ocotes, 20°39′N, 98°26′W, 1750 m, 2 Oct. 1980, *J. García P. 177* (holotype, XAL not seen; isotype, TEX not seen, TEX digital image TEX!).

Shrubs or trees, ca. 3 m tall; new growth densely velvety tomentose; stems terete, grey-green. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 0.8–1.5 cm, tomentose; blades 10–15 × 1.5–2.3 cm, pinnate to pinnipalmate, oblong, apex acute, base cuneate, margins subentire, weakly callous denticulate, in-rolled; abaxially glabrate to light velvety tomentose, adaxially glabrous, dark green. *Capitulescence* pyramidal compound paniculate cyme, 10 to 20 capitula per branch, peduncles velutinous, primary bracts linear, ca. 1 mm, ultimate peduncles 1–4 mm, bracteoles 0 to 2, scale-like, ca. 1 mm. *Capitula* radiate, ca. 8 × 2.5 mm, turbinate, corollas yellow; calyculate bracts 3 to 6, velutinous, linear, ca. 1 mm; phyllaries 11 to 14, glabrous to slightly pubescent, purple-tinted, 4–5 × ca. 1 mm. Ray florets ca. 8, corolla ligulate, glabrous, tube ca. 4.5 mm, ligule ca. 4.5 × 2 mm. Disk florets 9 to 11, corolla narrow, glabrous, ca. 7 mm, tube ca. 3 mm, throat ca. 3 mm, lobes ca. 1 mm. Achenes glabrous, columnar, ca. 2 mm, ribs 5 or 10, resin glands absent; pappus bristles 5–6 mm.

Distribution and phenology. Eastern Mexico (Hidalgo, Puebla, Veracruz) at elevations of 1800–2000 m. Found in pine-oak forests. Flowering November through December.

Comments. *Roldana neogibsonii* is a unique and easily recognized species. It has a persistent velvety tomentum on its stems, on the undersides of the leaf blades, and in the capitulescence. Its leaf blades are up to 5× longer than wide with subentire in-rolled margins; the callous denticles may require a hand lens to see.

Representative specimens examined. MEXICO. **Hidalgo:** Zacualtipán, 1800 m, Jan. 1940, *Martínez s.n.* (F); Zacualtipán, 1800 m, 15 Dec. 1939, *Martínez s.n.* (GH). **Puebla:** mtns. W of Huauchinango, 1 Nov. 1943, *Lundell 12633* (MICH); Puente Madera, SE of Tepehuaquila, rd. to Zacatepec, mpio. Huauchinango, 20°5′N, 98°2′W, 2000 m, 26 Feb. 1987, *Tenorio L. 12688* (KSC). **Veracruz:** mpio. Huayacocotla, orilla del camino entre Helechales y Ocotes, 20°39′N, 98°26′W, 1750 m, 2 Oct. 1980, *J. García P. 177* (TEX digital image TEX).

37. Roldana petasitis (Sims) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Cineraria petasitis* Sims, Bot. Mag. 37: t. 1536. 1813. *Senecio petasitis* (Sims) DC., Prod. 6: 431. 1837 [1838]. TYPE: same as that of *Senecio lobatus* Sessé & Moc. (neotype, designated here, F!).

Cineraria platanifolia Schrank, Pl. Rar. Hort. Monac. 2: t. 95. 1819 [1821]. TYPE: same as that of *Senecio lobatus* Sessé & Moc. (neotype, designated here, F!).

Senecio lobatus Sessé & Moc., Fl. Mexic. (ed. 2) 185. 1894, nom. illeg., non *Senecio lobatus* Pers., 1807. TYPE: Mexico. [s. loc.], *Sessé & Mociño 3152 bis* (lectotype, designated here, F!, F photo MICH 1763!).

Senecio petasioides Greenm. in J. D. Smith, Bot. Gaz. 37: 419. 1904. Syn. nov. *Roldana petasioides* (Greenm.) H. Rob., Phytologia 32: 331. 1975. TYPE: Guatemala. Santa Rosa: Cenaguilla, 4000 ft., Feb. 1892, *Heyde et Lux. 4522* (holotype, GH!; isotypes, F!, MO!, NY!, US not seen).

Senecio prainianus A. Berger, Gard. Chron. 3: 82. 1911. Syn. nov. TYPE: same as that of *Senecio lobatus* Sessé & Moc. (neotype, designated here, F!).

Suffruticose herbs or shrublets, 0.5–3 m tall; new growth densely pubescent with stipitate-glandular and

long multicelled hairs on stems and lanate tomentose on abaxial leaf surfaces; stems terete, maculate, dark red to greenish brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 2–14 cm, variously pubescent with glandular hairs; blades 5–23 × 5–23 cm, thick or at times membranous textured, triple-nerved, shape of two types: variously orbicular to subovate, 7- to 9-lobed, sinus depth about 1/8 of the way to the midrib or less, lobes acute to rounded; or subpalmatifid, 5- to 7-lobed, sinus depth about 1/2 of the way to the midrib, lobes acute; abaxially variously glabrescent, pubescent, or tomentose, adaxially glabrous to pubescent with short glandular hairs. *Capitulescence* compound paniculate cyme, 10 to 60 capitula per branch, peduncles densely stipitate-glandular pubescent, primary bracts linear, 5–15 mm or obovate, 10 30 × 5–15 mm, ultimate peduncles 8 20 mm, bracteoles 0 to 3, filiform, up to 3 mm. *Capitula* eradiate or radiate, 6–16 × 2–4 mm, campanulate or turbinate, corollas yellow; calyculate bracts 0 to 3, linear, 1–2 mm; phyllaries ca. 8, densely stipitate-glandular pubescent, 3–9 × 1–2 mm. Ray florets absent or 3 to 8, corolla ligulate or reduced to a tube, glabrous, tube 4–6 mm, ligule 2 -10 × 1–4 mm. Disk florets 5 to 15, corolla campanulate, glabrous, 6–11 mm, tube 3–5 mm, throat 3–5 mm, lobes ca. 2 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 6–8 mm.

Distribution and phenology. Mexico, Guatemala, El Salvador, Honduras, and Nicaragua (El Salvador: Ahuachapán, Chalatenango, San Salvador, Santa Ana, San Vicente, Sonsonate; Guatemala: Alta Verapaz, Baja Verapaz, Chimaltenango, El Progreso, Guatemala, Huehuetenango, Jalapa, Jutiapa, Quezaltenango, San Marcos, Sololá, Totonicapán, Zacapa; Honduras: Comayagua, Copán, El Paraíso, Intibucá, La Paz, Lempira, Morazán, Ocotepeque; Mexico: Chiapas, Hidalgo, México, Oaxaca, Puebla, Tamaulipas, Veracruz; Nicaragua: Estelí, Madriz) at elevations of 1000–2500 m. Found in montane cloud forests and pine-oak forests with *Pinus*, *Quercus*, *Liquidambar*, *Ulmus* L., *Weinmannia* L., and *Styrax*. Flowering December through April.

A single naturalized roadside population in Victoria, Australia, is reported in *Flora of Victoria* (ed. D. B. Forman & N. G. Walsh, 4: 968, f. 198d. 1999).

Comments. The typification of *Roldana petasitis* is confounded by the fact that it is a pretty plant; several names were described from horticulturally tended plants that were never collected for specimens. The names without type material are *Cineraria petasitis* Sims, *C. platanifolia* Schrank, and *Senecio prainianus* A. Berger. The details concerning the search for type material are listed below. In the protologue of *S. lobatus*, Sessé and Mociño (1894) did not list any specimens; the lectotype was chosen from original Sessé and Mociño material at F. The same specimen is designated here as the neotype of *C. petasitis*, *C. platanifolia*, and *S. prainianus*, thereby making the four names homotypic.

Cineraria petasitis Sims: based on a plant grown in England by A. B. Lambert, blooming in Dec. 1812. No material found at G by Laurent Gautier (pers. comm., Oct. 2004). No material found at BM or K by Peta Hinds (pers. comm., Nov. 2004).

Cineraria platanifolia Schrank: no material found at K by Nicholas Hind (pers. comm., Oct. 2004). No material found that was definitely collected by Schrank at M by Franz Schuhwerk (pers. comm., Oct. 2004).

Senecio prainianus A. Berger: based on cultivated plants "Mexico, in montibus supra Vera Cruz; frutex a cl. A. Purpus lectus et in Hortulum cl. C. Sprenger introductus, primum floret in Horto Mortolensi mensibus, Febr.–April, 1910." No herbarium gathering is noted.

Roldana petasitis is a beautiful and common species. It has been widely collected and grown in cultivation. It typically has a lanate tomentum on the abaxial leaf surface; the tomentum often with a silky appearance. At its northern-most distribution in central Mexico, the tomentum is lost from the abaxial surface and the leaves are membranous in texture. In Oaxaca and Veracruz, some populations have developed a more palmatifid leaf, which also lacks a tomentum and has smaller capitula. Plants in Oaxaca and Chiapas, Mexico, and Guatemala sometimes have smaller eradiate capitula and abaxial leaf surfaces only at most lightly tomentose.

In the past, several species have been circumscribed based on the above pattern. However, when viewing this complex of plants as a whole, the gross morphology makes it apparent that they are all of the same species. As one species, the morphological variability from north to south is recognized at the level of varieties.

The long list of synonyms for *Roldana petasitis* is probably due to its ubiquity. *Cineraria platanifolia* and *Senecio lobatus* have traditionally been placed in synonymy of *S. petasitis*, and I concur with their placement here. *Senecio prainianus* was part of a group of plants collected by A. Purpus in the barrancos above Veracruz. The specimens were apparently grown from seed by Berger at a garden in England. He was unable to identify his plant but stated that it resembled *R. petasitis*. His protologue also reads as a very fine description of *R. petasitis*, and so the name is placed in synonymy. *Senecio petasioides*

was described by Greenman in Smith (1904) as being different from *R. petasitis* due to its involucral bracts being glandular-hirsute and the heads being shorter. In comparing the type material of *S. petasioides* with other collections of *R. petasitis*, I have found that the pubescence of *S. petasioides* is not distinct and that the capitula size falls within the range of the species. Robinson and Brettell (1974) created the species *R. chiapensis*, stating that it differs from *R. cristobalensis* in the presence of rays. While this is correct, it does not separate it from *R. petasitis*. From examining the type material, I can find no distinction between *R. chiapensis* and *R. petasitis*.

KEY TO THE VARIETIES OF *ROLDANA PETASITIS*

1a. Capitula eradiate . . 37b. *R. petasitis* var. *cristobalensis*
1b. Capitula radiate, ray floret may be reduced to a tube.
  2a. Leaf blade palmatifid, 5- to 7-lobed . . . . . 37c. *R. petasitis* var. *oaxacana*
  2b. Leaf blade orbicular to ovate and 7(+)-lobed.
    3a. Abaxial leaf surface glabrescent to lightly tomentose . . . . . 37d. *R. petasitis* var. *sartorii*
    3b. Abaxial leaf surface tomentose to densely lanate tomentose . . . . . 37a. *R. petasitis* var. *petasitis*

37a. Roldana petasitis var. **petasitis**

Distinguishing characters. *Leaf* blade variously orbicular to subovate, 7- to 9-lobed, sinus depth about 1/8 of the way to the midrib or less, lobes acute to rounded; abaxially tomentose to densely lanate tomentose, adaxially pubescent with short glandular hairs. *Capitula* radiate, 10–16 × 2–4 mm, phyllaries ca. 8, densely stipitate-glandular pubescent, 7–9 × 1–2 mm. Ray florets 5 to 8, corolla ligulate, glabrous, tube ca. 6 mm, ligule 7–10 × 2–4 mm. Disk florets 10 to 15, 8–11 mm, tube 3–5 mm, throat ca. 5 mm, lobes ca. 2 mm. Pappus bristles 6–8 mm.

Distribution and phenology. Mexico, Guatemala, El Salvador, Honduras, and Nicaragua (El Salvador: Ahuachapán, Chalatenango, San Salvador, Santa Ana, San Vicente, Sonsonate; Guatemala: Alta Verapaz, Baja Verapaz, Chimaltenango, El Progreso, Guatemala, Huehuetenango, Jalapa, Jutiapa, Quezaltenango, Sacatepéquez, San Marcos, Santa, Rosa, Sololá, Totonicapán, Zacapa; Honduras: Comayagua, Copán, El Paraíso, Estelí, Intibucá, La Paz, Lempira, Morazán, Ocotepeque; Mexico: Oaxaca, Veracruz; Nicaragua: Jinotega, Madriz) at elevations of 1000–2500 m. Found in montane cloud forests and pine-oak forests with *Pinus*, *Quercus*, *Liquidambar*, *Ulmus*, *Weinmannia*, and *Styrax*. Flowering December through April.

Representative specimens examined. EL SALVADOR. **Ahuachapán:** W slopes Sierra de Apaneca, vic. of Apaneca, 1400–1600 m, 24 Jan. 1947, *P. C. Standley 2991* (F). **Chalatenango:** near summit of Los Esesmiles, 14°21′N, 89°9′W, ca. 2580 m, 10 Mar. 1942, *J. M. Tucker 987b* (F, MICH). **San Salvador:** Volcán de San Salvador, rd. from Finca Florencia to the S rim of the crater, 1680–1860 m, 30 Jan. 1946, *M. C. Carlson 379* (F). **Santa Ana:** Cerro Miramundo, above Hacienda Los Planos, NE of Meapan, 1890–2400 m, 25 Feb. 1946, *M. C. Carlson 947* (F). **San Vicente:** Volcán de San Vicente, 1200–1500 m, 7–8 Mar. 1922, *P. C. Standley 21553* (GH, NY). **Sonsonate:** San Juan de Dios, 1470 m, 27 Feb. 1907, *H. Pittier 1997* (F). GUATEMALA. **Alta Verapaz:** Pastizales cenagosos a la margen del Río Frio, entre Tactic y Santa Cruz, 1460 m, 14 May 1963, *A. Molina R. 12231* (NY). **Baja Verapaz:** dry rocky hills N of Santa Rosa, 30 Mar. 1939, *P. C. Standley 69758* (F). **Chimaltenango:** Barranca de La Sierra, SE of Patzum, 2100 m, 31 Dec. 1938, *P. C. Standley 61621* (F). **El Progreso:** hills betw. Finca Piamonte & slopes SE of Finca Piamonte, 2400–2500 m, 4 Feb. 1942, *J. A. Steyermark 43429* (F). **Guatemala:** on hills rd. betw. Guatemala & San Raimundo, 1650–1950 m, 16 Jan. 1939, *P. C. Standley 62997* (F). **Huehuetenango:** mtns. W of Aguacatan, on the rd. to Huehuetenango, ca. 1950 m, 27 Dec. 1940, *P. C. Standley 81281* (F). **Jalapa:** mtns. along the rd. betw. Jalapa & Paraiso, 1400–1700 m, 14 Nov. 1940, *P. C. Standley 77292* (F). **Jutiapa:** Volcán Suchitan, NW of Asuncíon Mita, 500–2050 m, 18 Nov. 1939, *J. A. Steyermark 31895* (F). **Quezaltenango:** slopes of Volcán de Zunil, Aguas Amargas, 2430–2850 m, 17 Feb. 1939, *P. C. Standley 65377* (F); region of Azufral, N slope of Volcán de Zunil, 2300–2500 m, 3 Feb. 1941, *P. C. Standley 85734* (F). **Sacatepéquez:** oak-pine woods along upper reaches of Río Sitio Nuevo betw. Santa Rosalia & first waterfall, 1200–1500 m, 9 Jan. 1942, *J. A. Steyermark 42232* (F). **San Marcos:** mtns. along National Route 1, ca. 2 mi. E of San Marcos, 2 July 1960, *R. M. King 3158* (MICH). **Santa Rosa:** Santa Rosa, Cenaguilla, 4000 ft., Feb. 1892, *Heyde et Lux 4522* (F, GH, MO, NY). **Sololá:** mixed forest area in Sierra Madre Mtns. near Nahuala, 2800 m, 20 Dec. 1972, *L. O. Williams 41423* (MICH). **Totonicapán:** along the rd. to Santa Cruz del Quiche, ca. 14 km generally NE of Totonicapán, ca. 7800 ft., 27 Jan. 1977, *R. M. King 7280* (MO [2576149, 2576297], NY). **Zacapa:** trail betw. Santa Rosalía de Mármol & Vegas, 19 Jan. 1942, *J. A. Steyermark 42959* (MO). HONDURAS. **Comayagua:** trail from Firea Sanson to Cerro Sanson, near Quaimaca, 18 Feb. 1955, *M. C. Carlson 3165* (F). **Copán:** 5 km SO de Santa Rosa de Copan, 1200 m, 29 Mar. 1963, *A. Molina R. 11679* (F, NY). **El Paraiso:** Cumbre on the hwy. leading from Guinope to Manzaragua, ca. 1440 m, 5 Jan. 1947, *P. C. Standley 2111* (F). **Estelí:** Faldas del Cerro El Tisey, 12°58′N, 86°22′W, 1400–1460 m, 6 Mar. 1982, *P. P. Moreno 15768* (MO). **Intibuca:** vic. of La Esperanza & Intibuca, 1500–1600 m, 31 Jan.–12 Feb. 1950, *P. C. Standley 25337* (F). **La Paz:** cloud forest area betw. Las Marías & Montaña Verde Cordillera, Guajiquiro, 1900 m, 6 Mar. 1969, *A. Molina R. 24053* (DS, F, NY). **Lempira:** Siquatepeque, N Campamento Naranjo, 11 km S Gracias, Parque Nacional de Celaque, 14°33′N, 88°40′W, 2470 m, 31 Jan. 1992, *Mejia Dario 3* (MO). **Morazan:** al NE de Valle Encantado, drainage of the Río Yeguare, ca. 87°W, 14°N, 1700 m, 23 Mar. 1966, *A. Molina R. 18373* (F, NY). **Ocotepeque:** about Belén Gualcho, 1500–2000 m, 2–15 Apr. 1977, *Alberto Rubio 57* (MO). MEXICO. **Oaxaca:** Cerro del Machete, Pachutla, 1 Feb. 1941, 1200 m, *Reko 6206* (F). **Veracruz:** Azumiate, Barranca de San Miguel Tlagguio-

tiopa, entre Tlacuiotiopa y Vagueria, 19 Jan. 1989, 2800 m, *Cházaro B. 5827* (WIS). NICARAGUA. **Jinotega:** in oak forest–cloud forest area, Cordillera Central de Nicaragua above & E of Jinotega, 1400–1600 m, 20 Feb. 1963, *L. O. Williams 24735* (F, NY). **Madriz:** Cerro El Fraile, 13°25′N, 86°16′W, 1100–1200 m, 10 Mar. 1984, *P. P. Moreno 23521* (MO).

37b. Roldana petasitis var. **cristobalensis** (Greenm.) Funston, comb. nov. Basionym: *Senecio cristobalensis* Greenm. in Loes., Bull. Herb. Boissier ser. 2. 6: 867. 1906. *Roldana cristobalensis* (Greenm.) H. Rob. & Brettell, Phytologia 27: 417. 1974. TYPE: Mexico. Chiapas, in distr. centrali, in fruticeto in valle Río Prospero in Hacienda Tierra colorada sita, *E. Seler 2106* (lectotype, designated here, GH!).

Distinguishing characters. *Leaf* blade orbicular to subovate, 7- to 9-lobed or occasionally subpalmatifid, 5 to 7 lobes, sinus depth about 1/2 of the way to the midrib, lobes acute; abaxially leaf surface variously pubescent to lightly tomentose, adaxially glabrate. *Capitula* discoid, ca. 6 × 2 mm, turbinate, phyllaries ca. 8, densely stipitate-glandular pubescent, 3–6 × ca. 1 mm. Disk florets 5 to 10, ca. 6 mm, tube ca. 3 mm, throat ca. 2 mm, lobes ca. 2 mm. Pappus bristles ca. 6 mm.

Distribution and phenology. Southern Mexico and Guatemala (Guatemala: Alta Verapaz, Huehuetenango, Jalapa, Quezaltenango; Mexico: Chiapas) at elevations of 1000–1600 m. Found in montane cloud forests. Flowering November through February.

Comments. Greenman in Loesener (1906) designated the holotype *E. Seler 2106* (B photos F!, GH!, MICH!, MO!, NY!) in the protologue. He also listed a paratype: Mexico. Chiapas, betw. San Cristóbal & Teopisca, 6700–8500 ft., 4 Dec. 1895, *E. W. Nelson 3469* (GH!, US!, B). The Berlin specimens of both collections were destroyed in the 1943 herbarium fire.

Roldana petasitis var. *cristobalensis* has many characters that overlap between the typical variety and variety *oaxacana*. Its leaf shape is usually that of variety *petasitis*, but on occasion it has the subpalmatifid form of variety *oaxacana*. The abaxial leaf pubescence is usually lightly to densely pubescent, but it can have a light tomentum. The capitula are the smaller turbinate capitula, although eradiate, of variety *oaxacana*.

Consideration was given to maintaining *Roldana cristobalensis* and/or *R. oaxacana* as species, but when taking into account the gross morphology and distribution of all the taxa involved and then the intermingling of characters, their recognition as varieties was deemed appropriate.

Representative specimens examined. GUATEMALA. **Alta Verapaz:** hills along Río Chio, ca. 2–4 km SW of Coban, 1300–1400 m, Jan.–Feb. 1969, *L. O. Williams 40725* (F, MICH, NY). **Huehuetenango:** above Malacatancito, 1900 m, 10 Jan. 1974, *A Molina R. 30219* (F, MICH). **Jalapa:** vic. of Soledad, Montaña Miramundo, betw. Jalapa & Mataquescuintla, 2000–2500 m, 4 Dec. 1939, *J. A. Steyermark 32641* (F). **Quezaltenango:** Fuentes Geoginas, western slope of Volcán de Zunil, ca. 2850 m, 4 Mar. 1939, *P. C. Standley 67498* (F). MEXICO. **Chiapas:** SW of Mexican hwy. 190 near Rancho Nuevo ca. 9 mi. SE of San Cristóbal de las Casas, mpio. San Cristóbal de las Casas, 9000 ft., 7 Nov. 1965, *D. E. Breedlove 14151* (DS, MICH, NY); Clinica Yerba Buena, 2 km NW of Pueblo Nuevo Solistahuacan, 5400 ft., 23–24 Jan. 1965, *P. H. Raven 19858* (F, MICH); shrubby slope in the Paraje of Yash'anal, mpio. Tenejapa, 6000 ft., 21 Feb. 1967, *A. S. Ton 2092* (DS, F, MICH, NY).

37c. Roldana petasitis var. **oaxacana** (Hemsl.) Funston, comb. nov. Basionym: *Senecio oaxacanus* Hemsl., Biol. Cent.-Amer., Bot. 2: 244. 1881. *Roldana oaxacana* (Hemsl.) H. Rob. & Brettell, Phytologia 27: 422. 1974. TYPE: Mexico. Oaxaca: Cordillera, 5500 ft., 1840, *H. Galeotti 2009* (holotype, K not seen; isotypes, F not seen, G not seen, GH!, NY not seen, US not seen, G photos F!, MICH!, MO!, GH photo MO!).

Senecio hederoides Greenm. in Loes., Bull. Herb. Boissier, ser. 2. 6: 868. 1906. Syn. nov. *Roldana hederoides* (Greenm.) H. Rob. & Brettell, Phytologia 27: 420. 1974. TYPE: Mexico. Oaxaca: near Reyes, 2000–3045 m, 20 Oct. 1894, *E. W. Nelson 1002* (lectotype, designated here, GH!; isotype, US!).

Senecio hypomalacus Greenm., Ann. Missouri. Bot. Gard. 1: 278, pl. 10. 1914. TYPE: Mexico. Oaxaca: mtns. of Telixtlahuaca, 7800 ft., 10 Dec. 1894, *Rev. Lucius C. Smith 368* (lectotype, designated here, GH!; isotype, GH fragm. & photo MO!).

Roldana chiapensis H. Rob. & Brettell, Phytologia 27: 416. 1974. Syn. nov. TYPE: Mexico. Chiapas: Mt. Pasitar, 31 Dec. 1936, *E. Matuda S-34* (holotype, US not seen, US photos F!, GH!, MO!; isotype, MICH!).

Distinguishing characters. *Leaf* blade subpalmatifid, 5 to 7 lobes, sinus depth about 1/2 of the way to the midrib, lobes acute; abaxially pubescent with stipitate-glandular and multicelled hairs, adaxially glabrate. *Capitula* radiate or disciform, ca. 6 × 2 mm, turbinate, phyllaries ca. 8, densely stipitate-glandular pubescent, 3–6 × ca. 1 mm. Ray florets ca. 3, corolla ligulate or reduced to a tube, glabrous, tube ca. 4 mm, ligule ca. 3 × 1 mm or when reduced tube ca. 2 mm. Disk florets 5 to 10, ca. 6 mm, tube ca. 3 mm, throat ca. 2 mm, lobes ca. 2 mm. Pappus bristles ca. 6 mm long.

Distribution and phenology. Southern Mexico (Chiapas, Oaxaca, Veracruz) at elevations of 1400–2000 m. Found in pine-oak forests. Flowering October through January.

Comments. In the protologue for *Senecio oaxacanus*, Hemsley (1881) cites the *Galeotti 2009* (K)

specimen as the holotype. The GH specimen has a note from Greenman, in hand, that it is of the type collection. The G specimen has a *Galeotti 2009* label and was determined by Greenman, in hand; however, it should be noted that the G photograph is seemingly of a different plant than the GH specimen, although undoubtedly a collection of *Roldana oaxacana*.

Regarding the typification of *Senecio hederoides*, Greenman in Loesener (1906) designated the holotype: Mexico. Oaxaca, distr. Nochiotlan, Cerro del Puebla Huautlilla, 15 Dec. 1895, *Seler 1571* (B photos F!, GH!, MICH!, MO!, NY!); however, the only known specimen was destroyed in the 1943 Berlin herbarium fire. The paratype *E. W. Nelson 1002* (GH) is chosen here as the lectotype. The label on the US specimen reads "E.W. Nelson 1802," a typographic error.

In the protologue for *Senecio hypomalacus*, Greenman (1914) cites *Rev. Lucious C. Smith 368* (GH, photo & fragm. MO) as the type material. The lectotype is chosen here.

Roldana petasitis var. *oaxacana* has a palmatifid form of leaf blade that distinguishes it from the typical variety. It also has smaller, turbinate capitula.

Gibson (1969) recognized *Senecio hederoides* as a distinct species, saying that it differs from *S. oaxacanus* in that the leaf blade lacks secondary lobing and prominent callous denticles. Greenman (1901) separates *S. hypomalacus* from *S. oaxacanus* by its having more distinctly lobed leaves that are thicker in texture and with a soft tomentum beneath. In comparing the type materials, I do not find the above combinations of characters distinguishable from the variation present in the collections for this taxon.

Representative specimens examined. MEXICO. **Chiapas:** mpio. Cintalapa, SE of Cerro Baul bordering Oaxaca, 16 km NW of Rizo de Oro along logging rd. to Colonia Figaron, 1600 m, 8 Jan. 1973, *D. E. Breedlove 31449* (MO). **Oaxaca:** on hwy. 175 N of Oaxaca, ca. 21 km N of jct. with hwy. 190, on first high ridge in mixed forest, 19 Oct. 1956, *T. M. Barkley 3904* (KSC, WIS); ranch of Boone Hallberg, ca. 5 km W of Ixtlan de Juarez, 17°18′N, 96°26′W, 2000 m, 20 Nov. 1982, *Benz 689* (KSC, WIS); 14 mi. NE of Tlacolula, 3100 m, 28 Oct. 1965, *A. Cronquist 10432* (CAS, F, GH, KANU, KSC, MICH, NY); hwy. 175, 12 mi. E of jct. of hwy. 190 in Oaxaca, 1 mi. W of La Cumbre, 9 Jan. 1983, *Spellenberg 6910* (NMC); S of El Limon el cual esta, 11.1 km al SW of jct. Tehuantepec and Buenos Aires, 9 Dec. 1983, *Torres C. 4289* (NY). **Veracruz:** Vic. Puente Acabaloya, ca. 5 km NW of Xico on trail to Xico Viejo, mpio. Xico (= Jico), 1600 m, 5 Feb. 1984, *M. Nee 29399* (KSC, NY).

37d. Roldana petasitis var. **sartorii** (Sch. Bip. ex Hemsl.) Funston, comb. nov. Basionym: *Senecio sartorii* Sch. Bip. ex Hemsl., Biol. Cent.-Amer., Bot. 2: 247. 1881. *Roldana sartorii* (Sch. Bip. ex Hemsl.) H. Rob. & Brettell, Phytologia 27: 423. 1974. TYPE: Mexico. Veracruz: Cordillera of Vera Cruz, 3000 ft., *Galeotti 2225* (lectotype, designated here, K not seen; isotype, GH!).

Distinguishing characters. *Leaf* blade variously orbicular to subovate, 7- to 9-lobed, sinus depth about 1/8 of the way to the midrib or less, lobes acute to rounded; usually with a membranous texture to blade; abaxial surface glabrescent to lightly tomentose, adaxially glabrous. *Capitula* radiate, 8–12 × ca. 2 mm. Ray florets 5 to 8, ligule 6–8 × ca. 2 mm. Disk florets 10 to 15, 7–10 mm, tube ca. 4 mm, throat ca. 3 mm, lobes ca. 2 mm. Pappus bristles 6–8 mm.

Distribution and phenology. Mexico (Hidalgo, México, Oaxaca, Puebla, Tamaulipas, Veracruz) at elevations of 1200–1800 m. Found in pine-oak forests. Flowering in January and February.

Comments. In the protologue, Hemsley (1881) listed three syntypes from southern Mexico: Cordillera of Vera Cruz, 3000 ft., *Galeotti 2225*; Mirador, *Linden 1164*; and, probably Mirador, *F. M. Liebmann 156*—all deposited at Kew. Having only seen the Galeotti gathering, it was chosen for lectotypification.

Roldana petasitis var. *sartorii* represents the northern extreme of the species *R. petasitis*. Much consideration was given to placing the taxon in synonymy; however, because its distribution is disjunct across the Isthmus of Tehuantepec and the difference in pubescence so extreme, it was given varietal rank.

Representative specimens examined. MEXICO. **Hidalgo:** 5 km S of Tianguistengo, 1800 m, 25 Mar. 1981, *Hernandez M. 5611* (CAS); Ropala & Tzincuatlan near rd. from Zacualtipán to Tlahuelompa, 2000 m, 7 Nov. 1946, *Moore Jr. 1885* (GH). **México:** Toluca, 3300 m, 18 Feb. 1931, *Hunnewell 11851* (GH). **Oaxaca:** along hwy. 175 betw. Oaxaca & Pochutla 77.8 mi. S of Miahuatlan, 20.3 mi. S of Suchixtepic, 9.9 mi. N of turnoff to Pluma Hidalgo, 1480 m, 20 Jan. 1979, *T. B. Croat 46075* (KSC, MO). **Puebla:** 6 km above rd. to Matitla, 12 km from entrance to Nauzontla, 1200 m, 30 Apr. 1983, *García P. 1752* (KSC); 6 mi. S of Villa Juarez, 1000 m, 23 Feb. 1961, *McGregor 16430* (KANU). **Tamaulipas:** 4 mi. W of Sierra Madre Oriental, vic. of Gomez Farias, 4600 ft., 27 May 1950, *B. E. Harrell 381* (MO). **Veracruz:** 1 km S of Palmillas, 19°13′N, 96°46′W, 600 m, 13 Mar. 1985, *Castillo C. 4267* (NY); 2.5 mi. N of La Joya on dirt rd., ca. 17 mi. NW of Jalapa, 7 Dec. 1974, *Stuessy 3667* (MICH, OS).

38. Roldana platanifolia (Benth.) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Senecio platanifolius* Benth., Pl. Hartw. 43. 1840. TYPE: Mexico. Michoacán: in pinetis, Chico, 1840, *Hartweg 331* (holotype, K not seen; isotypes, G not seen, NY!, G photos F!, MICH!).

Perennial herbs, acaulescent to subcaulescent, 0.4–1 m tall, arising from a slender woody rhizome (not bulbous); plants densely pubescent with a mixture of

stipitate-glandular and long multicelled hairs, stems cream with patches of purple. *Leaves* clustered at base; petioles 6–12 cm, densely pubescent; blades 6–11 × 8–13 cm, triple-nerved, palmatifid, 5- to 7-lobed, sinus depth about 1/2 of the way to the midrib, lobes acute secondarily lobed, callous denticulate; surfaces glabrate, sparsely to densely pubescent along veins. *Capitulescence* a terminal simple paniculate cyme borne atop a naked scape, 20–40 cm, or a terminal compound paniculate cyme arising from an obvious stem, 6 to 20 capitula per capitulescence, scape and peduncles stipitate-glandular pubescent, primary bracts linear, 4–10 mm, ultimate peduncles 8–20 mm, bracteoles 0 to 3, linear to filiform, 2–5 mm. *Capitula* radiate, 12–15 × 4–6 mm, campanulate, corollas yellow; calyculate bracts 0 to 3, linear or filiform, ca. 2 mm; phyllaries ca. 13, densely stipitate-glandular to glabrate, ciliate hairs on margins, greenish 8–13 × ca. 2 mm. Ray florets ca. 8, corolla ligulate, glabrous, tube 5–8 mm, ligule 9–14 × 3–5 mm. Disk florets 30 to 40, corolla funnelform, glabrous, 7–11 mm, tube 4–5 mm, throat ca. 5 mm, lobes ca. 1 mm. Achenes glabrous, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles 9–11 mm.

Distribution and phenology. Central Mexico (Distrito Federal, Hidalgo, México, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla) at elevations of 2700–4100 m. Found in pine-oak and fir forests. Flowering October through February.

Comments. Roldana platanifolia is the most widely distributed of the smaller herbs in the genus. Its palmately lobed leaf blade is very similar to *R. angulifolia*. Its coloration is a somewhat unique creamy green with purple hues throughout. The dense pubescence of mixed stipitate-glandular and long multicelled hairs also distinguishes this species.

Representative specimens examined. MEXICO. **Distrito Federal:** Parque Nacional, Desierto de los Leones, 3000 m, 28 Nov. 1971, *Barrios 19* (KANU, MICH, UMO, WIS). **Hidalgo:** below Parque Nacional El Chico on rd. from Real del Monte to El Chico, 3000 m, 27 Oct. 1949, *Moore Jr. 5426* (MICH). **México:** N slope of Volcán Popocatépetl, 3300 m, 29 Oct. 1980, *A. Cronquist 11751* (GH, KSC, NY); on forestry rd. 2–4 km from hwy., 41 km N of Temescaltepec to turn off for hwy. 134, 19°10′33″N, 99°52′18″W, 2805 m, 24 Mar. 1996, *T. M. Barkley 4031* (KSC, MO). **Michoacán:** rocky open peak on E mtn. slope, 3100 m, 13 Aug. 1961, *DeJong 1057* (MICH). **Morelos:** Parque Nacional de Zempoala, 19 km E of Tres Marías, 2960 m, 10 Feb. 1979, *García P. s.n.* (MICH). **Nuevo León:** Zaragoza, Cerro del Viejo, 15 mi. W Dulces Nombres, 19 Aug. 1948, *F. G. Meyer 3011* (MO). **Oaxaca:** Uxpanapa Region, along gravel rd. from Esmeralda to Rio Verde, 1.1 mi. S of Esmeralda, 17 10′N, 94 45′W, 100 m, 18 Jan. 1987, *T. B. Croat 65784* (MO). **Puebla:** Mt. Popocatepetl, 4100 m, 9 Feb. 1965, *E. S. Gibson 996* (KSC).

39. Roldana reglensis (Greenm.) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Senecio reglensis* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 283. 1907. TYPE: Mexico. Veracruz: Regla, Sept.–Oct. (s.d.), *C. Ehrenberg 454* (holotype, B fragm. & tracing GH!, B photos F!, GH!, MICH!, MO!, B tracing MO!)

Single-stemmed perennial herbs, 1–2.5 m tall; plants glabrate to weakly pubescent with hirsute hairs; stems terete. *Leaves* cauline, evenly distributed on stem; blades 5–7 × 5–7 cm, blades palmatifid, 5- to 7-lobed, sinus depth about 1/2 of the way to the midrib, lobes acute, weakly secondarily lobed to denticulate; abaxially hirsute-pubescent, adaxially glabrous. *Capitulescence* a rounded compound paniculate cyme, 10 to 20 capitula per branch; calyculate bracts ca. 5, linear, 1–2 mm. *Capitula* radiate, 9–12 × 3 mm, campanulate. Ray florets ca. 6. Disk florets ca. 20. Achenes glabrous. (Description compiled from the literature.)

Distribution. Mexico (Veracruz) at elevations ca. 1200 m. Greenman (1907) lists the type locality as "Regla, Vera Cruz." However, that city is in Hidalgo, not far from the Veracruz border, making the collection locality somewhat unclear.

Comments. Greenman (1907) designated the type *C. Ehrenberg 454* (GH) in the protologue. The GH specimen is a fragment and tracing of a Berlin specimen that was destroyed in 1943. Comments made by Greenman (1914: 279) in the protologue of *Senecio kerberi* make it known that the GH fragments came from the Berlin type collections. Also, it is apparent that the GH tracing done by Greenman is of the B specimen that is depicted in all of the photographs, as well as the MO tracing. Therefore, the GH fragment and tracing are determined here to be the holotype.

Roldana reglensis is seemingly known only from a fragment, tracings, and photographs. It superficially resembles *R. mexicana* but has radiate capitula. It is provisionally accepted.

40. Roldana reticulata (DC.) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Senecio reticulatus* DC., Prodr. 6: 431. 1837 [1838], non *Senecio reticulatus* C. B. Clarke, 1876. TYPE: Mexico. [state unknown]: Villalpando, *Mendez 18* (holotype, G-DC not seen, microfiche IDC 800. 1142 II 8!, G-DC photos F!, MICH!, MO!; isotypes, G-DC not seen, G-DC microfiche IDC 800. 1142 III 1–3(pp)!).

Senecio dictyophyllus Benth., Pl. Hartw. 43. 1840. TYPE: Mexico. Hidalgo: Guajolote near Real del Monte,

Hartweg 327 (holotype, K not seen, K photos F!, MICH!; isotype, NY!).

Single-stemmed perennial herbs, 0.5–1 m tall, much branched above, arising from a woody caudex; plants essentially glabrous; stems terete, striated, cream to red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 0.5–1.5 cm, glabrous; blades 4–8 × 4–8 cm, triple-nerved, ovate, 5- to 12-lobed, sinus depth about 1/8 of the way to the midrib, lobes acute, may be secondarily lobed, giving margins an irregularly serrate appearance to merely subentire and denticulate, surfaces glabrous, margins may be sparsely pubescent with short glandular hairs. *Capitulescence* flat-topped or rounded compound corymbiform cyme, 3 to 20 capitula per branch, peduncles glabrous, some plants with tufts of arachnoid pubescence at base of capitula, primary bracts linear-oblong, to 3.5 cm, ultimate peduncles 5–25 mm, bracteoles 0 to 3, linear, 1–2 mm. *Capitula* radiate, 8–15 × 5–10 mm, campanulate, corollas yellow to yellow-orange; calyculate bracts 3 to 6, linear to obovate, 2–13 × 1–5 mm; phyllaries 9 to 13, glabrous, greenish, some purple-tipped, 7–12 × 1–2 mm. Ray florets 5 to 8, corolla ligulate, glabrous, tube 4–6 mm, ligule 8–15 × ca. 3 mm. Disk florets 19 to 37, corolla funnelform, glabrous, 7–11 mm, tube 3–4 mm, throat 3–5 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles 5–9 mm.

Distribution and phenology. Central Mexico (Distrito Federal, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Puebla, Querétaro) at elevations of 2800–3600 m. Found in pine-oak forests. Flowering September through December.

Comments. The most distinguishing feature of *Roldana reticulata* is its rather small ovate leaf blades, which are attached to the stem by a short petiole and have a short internodal distance. When pressed, these characters combine to appress the leaf blades adaxially to the stem essentially covering the stem from view. It is also one of the few species in the genus with a corymbiform cyme. Upon examining the protologue and type material for *Senecio dictyophyllus*, it is placed in synonymy.

Representative specimens examined. MEXICO. **Distrito Federal:** Cerro El Tepalcate, about Puerto de Las Cruces, Sierra de Las Cruces, 3100 m, 19 Nov. 1965, *J. Rzedowski 21728* (DS, F, GH, MICH, UC, US, WIS). **Guanajuato:** ca. 3 km E of Santa Rosa, 2600 m, 11 Nov. 1970, *R. McVaugh 24218* (MICH). **Guerrero:** Teotepec, distr. Galeana, 3200 m, 26 Dec. 1937, *G. B. Hinton 11136* (GH, MICH, UC). **Hidalgo:** parte alta del Cerro Xihuingo, mpio. Tepeapulco, 3000 m, 7 Oct. 1973, *J. Rzedowski 31478* (MICH, UMO). **Jalisco:** Volcán Tequila, rd. to microwave station, ca. 20°47′N, 103°50′W, 2800 m, 24 Oct. 1970, *Webster 15914* (DAV, MICH, MO). **México:** just S of Pan-Am hwy. at Pass of Monte Río Frio, 5 km WNW of Río Frio at Llano Grande, 19°22′N, 98°43′W, 3100 m, 18 Aug. 1960, *H. H. Iltis 1090* (WIS). **Michoacán:** Tancítaro, Uruapan, 3800 m, 27 Oct. 1940, *G. B. Hinton 15595* (GH, NY); Mt. Tancítaro, *Leavenworth 1165* (F, MO). **Morelos:** Lagunas de Zempoala, 1 Oct. 1932, *Lyonnet 797* (CAS). **Puebla:** Ixtaccíhuatl above Huejotzingo, 4000 m, 21 Oct. 1945, *Hernandez X. 139* (KSC). **Querétaro:** mpio. Colón, 1 km SW of la cumbre, Cerro Zamorano, 3100 m, 13 Nov. 1971, *J. Rzedowski 417* (CAS, MICH).

41. Roldana robinsoniana (Greenm.) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Senecio robinsonianus* Greenm. in Sarg., Trees & Shrubs 1: 19. 1902. TYPE: Mexico. Oaxaca: betw. Nopala & Mixistepec, 5 Mar. 1895, *E. W. Nelson 2439* (holotype, GH!; isotype, MO!).

Simple or sparingly branched herbs or subshrubs, 1–3 m tall; new growth covered with a dense white lanate tomentum; stems terete, light brown. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 5–10 cm, tomentose; blades 10–15 × 10–15 cm, palmate- to triple-nerved, ovate to cordate, 5(+)-lobed, sinus depth less than 1/8 of the way to the midrib or merely denticulate; abaxially densely pubescent to lanate tomentose and canescent, adaxially glabrous to sparsely pubescent with short glandular hairs. *Capitulescence* compound paniculate cyme terminating in glomerules or short pedicellate cymules, 30 to 60 capitula per branch, peduncles lanate, primary bracts linear, ca. 4 mm, ultimate peduncles 0–2(–4) mm, bracteoles 0 to 3, filiform, ca. 1 mm. *Capitula* radiate, 6–8 × 2–3 mm, turbinate, corollas yellow; calyculate bracts ca. 5, filiform, 1–2 mm; phyllaries ca. 8, glabrous to arachnoid pubescent, purple-tinted, 3–5 × ca. 1 mm. Ray florets ca. 5, corolla ligulate, glabrous, tube 2–4 mm, ligule 2–4 × ca. 1 mm. Disk florets 7 to 13, corolla funnelform to moderately campanulate, glabrous, 4–7 mm, tube 2–3 mm, throat 1–2 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, 0.5–1 mm, ribs 5, resin glands absent; pappus bristles 4–6 mm.

Distribution and phenology. Southwestern Mexico (Jalisco, Michoacán, Oaxaca) at elevations of 1200–1900 m. Pacific slopes, tropical deciduous and lower pine-oak forests with *Podocarpus*, *Distylium*, *Ostrya*, *Quercus*, and *Pinus*. Flowering December through March.

Comments. *Roldana robinsoniana* is a poorly collected species from southwestern Mexico. It most closely resembles *R. lobata* in that it has an elongate panicle that may terminate in glomerules and has a persistent tomentose pubescence.

Representative specimens examined. MEXICO. **Jalisco:** 30–35 km SE of Autlán, seaward-facing slopes, 1–4 km below summit, La Cumbre, near lumber rd. betw. El Chante & Cuzalapa, & above the abandoned site of Durazno, 19°32′N, 104°14′W, *R. McVaugh 23226* (MICH). **Michoacán:** *Langlasse 901* (GH). **Oaxaca:** 17 km NE of Piedra Larga, rd. Piedra Larga–Miahuatlan, *Martínez S. 2733* (KSC); ca. 80 km SSW of Sola de Vega, seaward side of pass 25 km above S. Gabriel Mixtepec & ca. 30 km S of the Río Verde crossing at Juchatengo, mpio. Juquila, *R. McVaugh 22373* (DS, MICH, NY).

42. Roldana scandens Poveda & Kappelle, Brenesia 37: 157–160. 1992 [1993]. TYPE: Costa Rica. Prov. San José: Reserva Forestal Los Santos, Cordillera de Talamanca, por el camino hacia San Gerardo de Dota cerca del caserió de Jaboncillos de Dota (Distrito de Copey), 2900 m, 26 ene. 1992 (fl.), *Kappelle 5843* (holotype, CR not seen; isotypes, ASD not seen, COL not seen, F not seen, MEXU not seen, MO!, NY not seen, U not seen, US not seen).

Shrubs, to 5 m tall; new growth glabrate to arachnoid floccose; stems terete, dark red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petiole 4–9 cm, glabrate; blades 4–9 × 5–14 cm, palmately nerved, orbicular, 9(+)-lobed, sinus depth 1/8 of the way to the midrib or less, lobes acute, weakly secondarily lobed to denticulate; surfaces glabrous at maturity, plush arachnoid when immature. *Capitulescence* pyramidal compound paniculate cyme, 30 to 70 capitula per branch, peduncles weakly arachnoid pubescent, primary bracts linear, 2–4 mm, ultimate peduncles 4–15 mm, bracteoles 1 to 2, linear, ca. 1 mm. *Capitula* radiate, 8–10 × 2–3 mm, turbinate, corollas yellow; calyculate bracts 3 to 5, linear, ca. 1 mm; phyllaries 5(to 6), glabrous, purple-tinted, 4–7 × 1–2 mm. Ray florets 2 to 3, corolla ligulate, glabrous, tube ca. 5 mm, ligule ca. 5 × 1–2 mm. Disk florets ca. 5, corolla funnelform, glabrous, 6–8 mm, tube ca. 3 mm, throat 1–2 mm, lobes 2–3 mm. Achenes glabrous, cylindrical, 1–2 mm, 5 ribs, resin glands absent; pappus bristles ca. 7 mm.

Distribution and phenology. Costa Rica (Puntarenas, San José) at elevations of 1800–3300 m. Found in open areas of oak forests. Collected in flower in September.

Comments. *Roldana scandens* is endemic to Costa Rica. It is best characterized by its palmately nerved, orbicular leaf blades. I affiliate it with *R. schaffneri* based on similarities in phyllary number (5 or 6), overall pubescence, capitulescence, and size of capitula.

Representative specimens examined. COSTA RICA. **Puntarenas:** Cordillera de Talamanca, upper slopes of Cerro Echandi, oak forest with Chusqa understory, 9°01′30″N, 82°49′00″W, 23 Aug. 1983, *Davidse et al. 23996* (MO). **San José:** high mtn. oak forest, on slopes & ridges, along the trail from Canaan to Chirripó via Los Angeles, above (N of) the Río Talari, 3100–3200 m, 9°30′N, 83°32′W, 19–22 Jan. 1970, *W. Cerro Burger 7433* (F); Cordillera de Talamanca, upper slopes, western ridge of Cerros Cuerici, trail along ridge with bamboo understory, ca. 9°34′N, 83°40′W, 3160 m, 15 Sep. 1983, *G. Davidse 24716* (KSC, MO); on slope in woods above cave, along Canaan–Valle de los Conegos (Chirripó massif), 3150–3300 m, 10 Dec. 1966, *A. S. Weston 3688* (UC).

43. Roldana schaffneri (Sch. Bip. ex Klatt) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Senecio schaffneri* Sch. Bip. ex Klatt, Leopoldina 24: 126. 1888. TYPE: Mexico. Veracruz: Mirador, *F. M. Liebmann* 151 (holotype, C-Liebmann not seen).

Senecio grandifolius Loes. var. *glabrior* Hemsl., Biol. Cent.-Amer., Bot. 2: 240. 1881. TYPE: Mexico. Veracruz: Orizaba, Valle Cordova, *E. Bourgeau 2207* (lectotype, designated here, K not seen; isotype, F!).

Senecio ghiesbreghtii Regel var. *pauciflorus* J. M. Coult., Bot. Gaz. 16: 101. 1891. *Senecio santarosae* Greenm., Publ. Field Columbian Mus., Bot. Ser. 2: 281. 1907, non *Senecio pauciflorus* Pursh, Fl. Amer. Sept. 2: 529. 1813. TYPE: Guatemala. Dept. Guatemala: Sapote, 1310 m, Mar. 1890, *J. D. Smith 2359* (lectotype, designated here, US-Donnell Smith not seen; isotypes, F!, GH not seen, MO!).

Senecio orogenes L. O. Williams, Phytologia 31: 443. 1975. Syn. nov. TYPE: Nicaragua. Dept. Matagalpa: Cordillera Central de Nicaragua, "Jinotega rock quarry," cloud forest area, 1500 m, 19 Feb. 1963, *L. O. Williams & T. P. Williams 24679* (holotype, F!; isotype, EAP not seen).

Suffruticose shrubs to small trees, 2–4 m tall; new growth arachnoid to tomentose; stems terete, dark red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 2–7 cm, glabrate with arachnoid tufts at axils; blades 12–50 × 5–9 cm, pinnate, elliptic to ovate, apex acute, base cuneate, margins subentire to serrate, or sinuately lobed; abaxially glabrate, weakly arachnoid pubescent on some veins, adaxially glabrous. *Capitulescence* pyramidal compound paniculate cyme, 30 to 50 capitula per branch, peduncles floccose arachnoid, primary bracts linear, ca. 2 mm; ultimate peduncles glabrate, 2–5 mm; bracteoles absent or scale-like, to 1 mm. *Capitula* radiate, 7–10 × ca. 2 mm, funnelform, corollas yellow; calyculate bracts ca. 3, linear, 1–2 mm; phyllaries 5 to 6, glabrate to floccose arachnoid, purple-tinted, 4–7 × 1–2 mm. Ray florets 3 to 5, corolla ligulate, glabrous, tube ca. 4 mm, ligule 3–5 × ca. 2 mm. Disk florets ca. 6, corolla funnelform, glabrous, 6–8 mm, tube 3–4 mm, throat 2–3 mm, lobes 1–2 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 4–6 mm.

Distribution and phenology. Southern Mexico, Guatemala, El Salvador, Honduras, and Nicaragua (El Salvador: La Libertad, San Salvador, Santa Ana; Guatemala: Alta Verapaz, Baja Verapaz, Chimaltenango, El Progreso, Guatemala, Huehuetenango, Jalapa, Quezaltenango, Sacatepéquez, San Marcos, Santa Rosa, Sololá, Suchitepéquez, Zacapa; Honduras: Copán, Intibucá, La Paz, Ocotepeque; Mexico: Chiapas, Guerrero, Oaxaca, Veracruz; Nicaragua: Estelí, Matagalpa) at elevations of 1300–1800 m. Found in pine-oak forests. Flowering April through February.

Comments. In the protologue for *Senecio schaffneri*, Klatt (1888) lists the paratype: Orizaba, *Thomas 1864* (C-Klatt not seen). In the protologue for *S. grandifolius* var. *glabrior*, Hemsley (1881) listed several syntypes housed at Kew: S Mexico, region of Orizaba, *Botter, 608, 819, 1102*; S Mexico, valley of Cordova, *Bourgeau 2207, 2335*; and Costa Rica, Volcan de Irazu, *Oersted s.n.*

In the protologue for *Senecio ghiesbreghtii* var. *pauciflorus*, Coulter (1891) lists two syntypes from Guatemala: Chucaneb, Dept. Alta Verapaz, 6000 ft., Apr. 1880, *J. D. Smith 1606*; and the chosen lectotype *J. D. Smith 2359*. While a herbarium abbreviation does not follow any of the specimens in the article, Coulter (1891: 95) states that he was asked to determine specimens for John Donnell Smith and that the "final and critical study of species" was done at Harvard University. While I have seen neither the US-Donnell Smith nor GH specimens, Greenman (1914) cites the gathering and these two specimens when he made the nomen novum *S. santarosae*, although he did not designate it as type material.

Roldana schaffneri is a unique species in the genus. It has pinnately veined leaf blades, the corolla lobes can be as long as the throat, and the pappus consistently exceeds phyllary height, giving the involucre a foreshortened appearance. These characteristics lead one to suspect an affinity with the genus *Digitacalia*. Within the genus, *R. schaffneri* is superficially similar to *R. hintonii* and *R. mezquitalana*. The latter two species have ca. 13 phyllaries versus the 5 to 6 phyllaries of *R. schaffneri*.

The numerous synonyms are a product of the species' wide variation of the leaf margin. The margins vary from subentire to serrate to having distinct sinuate lobes. Otherwise, these synonymized species all resemble *Roldana schaffneri*. However, a correlation between leaf margin type, distribution, and other morphological characters may be worth further investigation.

Representative specimens examined. EL SALVADOR. **La Libertad:** El Boqueron, Volcán San Salvador, 1887 m, 24 Feb. 1968, *A. Molina R. 21663* (F). **San Salvador:** Volcán de San Salvador, from Finca Las Brumas, 1680–2010 m, 3 Feb. 1946, *M. C. Carlson 465* (F). **Santa Ana:** Hacienda San Miguel near Metapan, chiefly on pine-forested slopes betw. Río San Miguel & summit of Cerro El Pinal, 600–1380 m, 22 Feb. 1946, *M. C. Carlson 780* (F). GUATEMALA. **Alta Verapaz:** along Quiche hwy. ca. 12 km W of San Cristóbal, ca. 1100 m, 24 Mar. 1941, *P. C. Standley 89728* (F, MO). **Baja Verapaz:** mtn. slopes N of divide N of Santa Rosa, 1650 m, 30 Mar. 1939, *P. C. Standley 69925* (F). **Chimaltenango:** rd. betw. Chimaltenango & San Martín Jilotepeque, 1500–1700 m, 22 Dec. 1940, *P. C. Standley 80924* (F). **El Progreso:** hills N of Finca Piamonte, betw. Finca Piamonte & summit of Volcán Santa Luisa, 2400–3333 m, 5 Feb. 1942, *J. A. Steyermark 43601* (F, GH). **Guatemala:** slopes of Volcán de Pacaya, betw. San Francisco Sales & the base of the active cone, 1800–2300 m, 20 Dec. 1940, *P. C. Standley 80522* (F). **Huehuetenango:** mtns. W of Aguacatan, on the rd. to Huehuetenango, ca. 1950 m, 27 Dec. 1940, *P. C. Standley 81207* (F). **Jalapa:** betw. Miramundo & summit of Montaña Miramundo, betw. Jalapa and Mataquescuintla, 6 mi. S of Miramundo, 2000–2500 m, 5 Dec. 1939, *J. A. Steyermark 32699* (F). **Quezaltenango:** along old rd. betw. Finca Pirineos & Patzulin, 1200–1400 m, 9 Feb. 1941, *P. C. Standley 86725* (F). **Sachitepéquez:** Volcán Atitlán, S side, 8100 ft., 14 Jan. 1935, *A. F. Skutch 2138* (A, F, NY). **San Marcos:** barrancas 6 mi. SW of town of Tajumulco, NW slopes of Volcán Tajumulco, 2300–2800 m, 26 Feb. 1940, *J. A. Steyermark 36671* (F). **Santa Rosa:** Santa Rosa or La Vega, 3000 m, Feb. 1893, *Heyde et Lux. 4520* (F, GH). **Sololá:** Volcán Santa Clara, S-facing slopes to summit, 2100–3000 m, 5 June 1942, *J. A. Steyermark 47039* (F). **Suchitepéquez:** Volcán Atitlán, S side, 8100 ft., 14 Jan. 1935, *A. F. Skutch 2138* (A, F, NY). **Zacapa:** trail betw. Santa Rosalia de Marmol & Vegas, 19 Jan. 1942, *J. A. Steyermark 42915* (F). HONDURAS. **Copán:** forest, Monte Grueso, above Ruinas de Copan, 8 May 1970, *M. Hernandez M. 5191* (GH). **Intibucá:** Baños Las Pilatas, La Esperanza, 2000 m, 3 Apr. 1956, *A. Molina R. 6227* (F). **La Paz:** betw. Las Marías & Montaña Verde Cordillera Guajiquiro, 1900 m, 6 Mar. 1969, *A. Molina R. 24049* (NY). **Ocotepeque:** along Yoroconte river betw. El Moral & Sinuapa, 1300 m, 10 Mar. 1969, *A. Molina R. 24189* (DS, F). MEXICO. **Chiapas:** montane rainforest at the E base of Cerro Tres Picos near Cerro Bola along a logging rd. SW of Colonia Agronomos Mexicanos, mpio. Villa Corzo, 1500–1800 m, 4 May 1972, *D. E. Breedlove 24959* (DS, MICH). **Guerrero:** 5 km SE of El Carrizal de los Bravos, rd. Filo de Caballo–Chichihualco, 2500 m, 21 Feb. 1983, *Martínez S. 3293* (NY). **Oaxaca:** San Juan Juquila, dist. Mixe, 1400 m, 24 Apr. 1983, *Cedillo 2244* (NY). **Veracruz:** Cerro de San Martín, Mapa 24.0, 59.5, *J. I. Calzada 217* (F, GH, MO); along winding rd. from Naolinco to Misantla, just above Santa Rita & 3 km by rd. N Paz de Enriquez, 19°50′N, 96°49′W, 1600 m, 2 Apr. 1983, *M. Nee 26409* (KSC, WIS). NICARAGUA. **Estelí:** Cerro Quiabú, 13 7′N, 86 26′W, 1604 m, 14 Jan. 1981, *P. Moreno 6016* (MO). **Matagalpa:** Cordillera Central de Nicaragua, "Jinotega rock quarry," cloud forest area, 1500 m, 19 Feb. 1963, *Williams 24679* (F).

44. Roldana sessilifolia (Hook. & Arn.) H. Rob. & Brettell, Phytologia 27: 423. 1974. *Cacalia sessilifolia* Hook. & Arn., Bot. Beechey Voy. 436. 1841. *Senecio beecheyanus* Sch. Bip., Flora. 28: 499. 1845, non *Senecio sessilifolius* Sch. Bip., Flora 28: 50. 1845, nom. illeg. superfl. *Senecio*

sessilifolius (Hook. & Arn.) Hemsl., Biol. Cent.-Amer., Bot. 2: 247. 1881, nom. illeg. *Pericalia sessilifolia* (Hook. & Arn.) Rydb., Bull. Torrey Bot. Club 51: 376. 1924. TYPE: Mexico. Nayarit: betw. San Blas & Tepic, ca. lat. N 22°, Dec. 1837, *Sinclair s.n.* (holotype, E-GL not seen; isotype, K not seen, K photos F!, MICH!).

Senecio ovatifolius Sch. Bip., Flora 28: 498. 1845. Syn. nov. *Cacalia cordifolia* Kunth, Nov. Gen. Sp. (folio ed.) 4: 131, t. 360. 1818, nom. illeg., non *Cacalia cordifolia* L. f., 351. 1781 [1782] nec *Senecio cordifolius* L. f., 372. 1781 [1782]. *Senecio cardiophyllus* Hemsl., Biol. Cent.-Amer., Bot. 2: 237. 1881, nom. superfl. *Pericalia ovatifolia* (Sch. Bip.) Rydb., Bull. Torrey Bot. Club 51: 377. 1924. TYPE: Mexico. México: Santa Rosa, 2377 m, Floret Sep. [1803]. *Humboldt & Bonpland s.n.* (holotype, P not seen, microfiche IDC 6209. 104 III 2!, P photos F!, MICH!, MO!, microfiche IDC 7440. 86 I 2!; isotype, K not seen).

Cacalia nutans Sessé & Moc., Pl. Nov. Hisp. 132. 1887. Syn. nov. TYPE: Mexico. México: Topelpa, near Mexico City, *Sessé & Mociño 2822* (lectotype, designated here, MA not seen, microfiche IDC BT13. 235 I 1!).

Single-stemmed perennial herbs, 0.5–2 m tall, arising from a bulbous caudex with woolly buds; plants glabrous; stems terete, striated, greenish to red in color. *Leaves* cauline, clustered at midstem to base; petioles 0–18 cm, glabrous; blades 8–18 × 9–20 cm, triple-nerved, reticulate, leaves may be sessile or petiolate, ovate, subcordate, 5- to 9-lobed, sinus about 1/8 of the way to the midrib, lobes acute, secondarily lobed to denticulate; surfaces glabrous, margins and veins rarely with multicelled hairs present. *Capitulescence* a simple to compound paniculate cyme, 2 to 6 capitula per branch, peduncles glabrous, primary bracts linear, up to 20 mm or on larger plants obovate, up to 40 mm, ultimate peduncles 5–220 mm, bracteoles 0 to 3, linear, 2–5 mm. *Capitula* eradiate, 10–25 × 5–10 mm, campanulate, corollas yellow-cream or white; calyculate bracts 5 to 10, linear, 2–10 mm; phyllaries ca. 13, glabrous, purple-tinted, 11–14 × 2–3 mm. Disk florets 40 to 70, corolla funnelform to slightly campanulate, glabrous, 10–12 mm, tube 5–7 mm, throat 2–3 mm, lobes 2–5 mm. Achenes glabrous, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 8–11 mm.

Distribution and phenology. Central Mexico (Aguascalientes, Chihuahua, Distrito Federal, Durango, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Nayarit, Querétaro, San Luis Potosí) at elevations of 1000–2900 m. Found in pine-oak forests with *Acacia* Mill., *Abies*, *Clethra*, *Piper* L., *Salvia*, *Fuchsia*, and *Dahlia*. Flowering August through February.

Comments. Regarding *Cacalia nutans*, Sessé and Mociño did not designate a type in the protologue. According to TL-2, the original Sessé and Mociño herbarium is at MA. In 1963, Pippen, in hand, chose *Sessé & Mociño 2822* from the material at MA as the lectotype. There are three other collections included in the microfiche material, IDC BT 13. 235 II-4: *Sessé & Mociño 2821*, *2829*, and *4118*. Type material of *Senecio ovatifolius* was destroyed in the 1943 Berlin herbarium fire.

Roldana sessilifolia, as its name implies, may have sessile leaf blades; however, this is not the typical condition. The following combination of characters distinguishes the species: eradiate capitula, terminal few-headed capitulescence with bracts linear, 2–4 cm long, and glabrous achenes.

Representative specimens examined. MEXICO. **Aguascalientes:** 21 mi. W of Aguascalientes, 2100 m, 3 Nov. 1959, *R. McVaugh 117* (MICH). **Chihuahua:** 1 mi. SW of Villa Matamoros, Sierra de Sta. Barbara, 2000 m, 1 Oct. 1959, *Correll 22791* (NY). **Distrito Federal:** bluffs of barranca above Santa Fé, 2600 m, *C. G. Pringle 7983* (F, GH). **Durango:** 13 km N of El Soldado along the rd. to Otinapa, 2100 m, 6 Oct. 1970, *A. Cronquist 10783* (NY); ca. 50 km W of Cerro Durango, near the hwy. to Mazatlán, 2550 m, 27 Sep. 1962, *R. McVaugh 21658* (MICH, NY). **Guanajuato:** 22 23 km W of Dolores Hidalgo, 2100 m, 9 Nov. 1970, *R. McVaugh 24093* (MICH). **Hidalgo:** roadside just before Apulco on rd. from Metepec to Agua Blanca, 2400 m, 7 Sep. 1948, *Moore Jr. 4889* (A, MICH). **Jalisco:** 28 km WNW of Huejuquilla El Alto, Jalisco, on rd. to Canoas, 2530 m, 21 Oct. 1983, *D. E. Breedlove 59068* (NY); 1 km SSW of Rincón de Manantlán, 13.5 km S of El Chante, 19 36′N, 104°13′W, 1510 m, 11 Oct. 1982, *H. H. Iltis 28807* (KSC, WIS); plateau center at 1–5 km NNE of Cerro San Miguel & 4 km S of Rincón de Manantlán rd. E toward Cerro Las Capillas from crossroads on plateau, Sierra de Manantlán Central, 19 33′35″N, 104 10′13″W, 2700 m, 13 Jan. 1980, *R. R. Koual 2850* (WIS). **México:** 4 km W of Coyotepec, de la Sierra Alcaparrosa, 2800 m, 16 Nov. 1980, *García P. 1376* (NY). **Michoacán:** rd. betw. Morelia & Pátzcuaro, 25 mi. W of Morelia, 2000 m, 23 Oct. 1960, *Pippen 48* (MICH). **Nayarit:** valley of Río Jesús María, ca. 10 km E of village Jesús María, W slope Sierra de Huichol, 1000 m, 20 Sep. 1960, *Feddema 1345* (MICH). **San Luis Potosí:** 27 mi. E of San Luis Potosí on the Río Verde hwy., 19 Sep. 1960, *Johnston 5642* (MICH).

45. Roldana subpeltata (Sch. Bip.) H. Rob. & Brettell, Phytologia 27: 424. 1974. *Senecio subpeltatus* Sch. Bip. in Seem., Bot. Voy. Herald 311. 1856. TYPE: Mexico. [state unknown]: Sierra Madre, *B. Seemann 1984* (holotype, GH!; isotypes, K not seen, P not seen, P photos F!, MICH!).

Sparingly branched perennial herbs, 1.5–2.5 m tall, arising from a rhizomatous caudex with fleshy innovations (not lanate on specimens examined); plants glabrous; stems terete, finely maculate, finely striate, cream with patches of purple. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 15–21 cm, glabrous; blades 18–

22 × 22.5–24 cm, eccentrically peltate, texture wax paper–like, triple-nerved, ovate to orbicular, 5- to 7-lobed, sinus depth less than 1/4 of the way to the midrib to merely denticulate, lobes acuminate; surfaces glabrous, abaxially paler than above. *Capitulescence* simple to compound paniculate cyme, axillary peduncles delicate, 3 to 12 capitula per branch, peduncles glabrous, primary bracts absent, ultimate peduncles 22–40 mm, bracteoles 0 to 3, scale-like, ca. 0.5 mm. *Capitula* eradiate, 15–20 × 3–5 mm, funnelform, corollas cream to white; calyculate bracts absent; phyllaries ca. 8, glabrous, purple-tinted at tips or throughout, ca. 15 × 1–2 mm. Disk florets ca. 12, corolla funnelform, pubescent with sparse small-clubbed multicellular hairs, ca. 14 mm, tube ca. 6 mm, throat ca. 7 mm, lobes ca. 1 mm. Achenes pubescent with sparse small hairs (difficult to see without magnification), cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles ca. 7–8 mm.

Distribution and phenology. Northwestern Mexico (Durango, Sinaloa) at elevations of 1800–2300 m. Found in moist ravines of pine forests. Flowering February through April.

Comments. McVaugh (1984: 841) discussed the locality from which the type collection was made, "probably came from [western Durango near the boundary of Sinaloa], or from somewhere between Durango and Tepic."

Roldana subpeltata is one of the few species with eccentrically peltate leaves. Other distinguishing features are the delicate lax peduncles of the capitulescence, the distinctly funnelform and eradiate capitula, and the wax paper–like texture of the leaf blades.

Representative specimens examined. MEXICO. **Durango:** along hwy. from Durango to Mazatlán, 50–54 mi. WSW of El Saltó, vic. of Rancho Los Angeles, near Sinaloa border, 2100 m, 25 Mar. 1951, *R. McVaugh 11587* (MICH); Mazatlán–Durango hwy., 3–15 km toward El Saltó from the Sinaloa boundary at El Palmito, 2000 m, 13 Apr. 1965, *R. McVaugh 23592* (DS, MICH, MO); mpio. El Saltó, along hwy. 40 from 1–4.6 mi. N of state line & 1.6–5.2 mi. S of Revolcaderos, ca. 3–4 mi. N of El Palmito, Sierra Madre Occidental, ca. 105°50′W, 23°36′N, 2200 m, 27 Mar. 1984, *A. C. Sanders 4950* (KSC). **Sinaloa:** just W of El Palmito, Rancho el Liebre, 2000 m, 12 Mar. 1980, *Lehto 24312* (ASU).

46. Roldana suffulta (Greenm.) H. Rob. & Brettell, Phytologia 27: 424. 1974. *Cacalia suffulta* Greenm., Proc. Amer. Acad. Arts 32: 310. 1897. *Pericalia suffulta* (Greenm.) Rydb., Bull. Torrey Bot. Club 51: 377. 1924. *Senecio suffultus* (Greenm.) McVaugh, Contr. Univ. Michigan Herb. 9: 477. 1972. TYPE: Mexico. Morelos: wet barranca above Cuernavaca, 1 Nov. 1896, *C. G. Pringle 6626* (holotype, GH not seen, GH photo MICH!; isotypes, F!, MO!, NY!).

Large single-stemmed perennial herbs, 1–3 m tall, spreading from a rhizomatous caudex with woolly buds; plants essentially glabrous, multicelled hairs may be dense on stem base; stems terete, striated, greenish to red. *Leaves* cauline, evenly distributed on upper half of stem, lowermost deciduous; petioles 8–20 cm, glabrous; blades 10–25(35) × 10–25(35) cm, ovate, 7- to 13-lobed, sinus depth about 1/8 of the way to the midrib, lobes acute, weakly secondarily lobed to denticulate; surfaces glabrous, margins and proximal veins with multicellular hairs present. *Capitulescence* flat-topped to rounded compound corymbiform cyme, 2 to 6 capitula per branch, peduncles glabrous, primary bracts obovate, up to 20 × 10 mm, ultimate peduncles 10–110 mm, bracteoles 0 to 3, obovate, 2–20 × 1–10 mm. *Capitula* eradiate, 10–27 × 5–10 mm. campanulate, corollas bright orange (yellow-orange to yellow); calyculate bracts ca. 5, oval-elliptic, enclosing the capitula, (3–8)10–25 × (2–5)8–15 mm; phyllaries ca. 13, glabrous, purple-tinted, 8–17 × 2–3 mm. Disk florets (29–47)90+, corolla funnelform, glabrous, (7.5–)12–16 mm, tube (3–)8–10 mm. throat 3–4 mm, lobes ca. 2 mm. Achenes pubescent, cylindrical, 2–3 mm, ribs 10, resin glands absent; pappus bristles 11–14 mm.

Distribution and phenology. Central Mexico (Durango, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nayarit) at elevations of 900–2400(–2900) m. Tropical deciduous and pine-oak forests. Flowering October through December.

Comments. *Roldana suffulta*'s large obovate calyculate bracts that envelop the capitula are emblematic of the species. Some of the specimens examined have smaller, fewer-flowered capitula and reduced obovate calyculate bracts. This form may be a hybrid between *R. suffulta* and *R. mexicana*.

Representative specimens examined. MEXICO. **Durango:** 41 mi. WSW of Durango along hwy. 40, 39.9 mi. E of Las Adjuntas, 27 Sep. 1984, *Sundberg 2892* (CAS). **Guerrero:** 15 km NE Puerto del Gallo, hwy. Atoyac, Filo de Caballo, mpio. Tlacotepec, 3100 m, 23 Nov. 1983, *Martínez S. 5633* (KSC). **Hidalgo:** mpio. Molango, 5–6 km N of Ismolintla, 1900 m, *R. Herbández M. 5910* (MO). **Jalisco:** 11 rd. mi. SW of Autlán & 2 rd. mi. SW of the pass, 1500 m, 1 Nov. 1962, *A. Cronquist 9774* (GH, MICH, MO, NY); trail to Las Joyas 3 km W of La Cumbre, 5 km SSW to WSW of Rincón de Mantlan, 16–18 km SSW of El Chante, 19 33–35′N, 104 14–16′W, 2000 m, 5 Jan. 1979, *H. H. Iltis 1210* (WIS). **México:** 1 km E of San Bártolo, Km 52.5 on Mex. hwy. 1 from Toluca to Valle de Bravo, 19 15′N, 100 4′W, 2300 m, 26 Nov. 1983, *Solheim 1004* (WIS). **Michoacán:** ca. 18 mi. E of Morelia, near Puerto Vicente Cortés Herrera, rte. 15 betw. Morelia & Cd. Hidalgo, 19°40′N, 100°57′W, 2200 m, 2 Nov. 1970, *Webster 16158* (MICH, UC). **Morelos:**

mtn. canyon above Cuernavaca, 11 Nov. 1902, 2000 m, *C. G. Pringle 9876* (F, GH, MICH, MO, NMC, NY). **Nayarit:** along hwy. 54 to Jalcocotan from hwy. 15 N of Tepic, 1000 m, 4 Nov. 1968, *Boutin 2041* (MICH); 8.6 mi. W of Rte. 15 on rd. to Jalcocotan, 1000 m, 6 Nov. 1977, *LaDuke 452* (MO).

47. Roldana sundbergii (B. L. Turner) B. L. Turner, Phytologia 80: 278. 1996. *Senecio sundbergii* B. L. Turner, Brittonia 37: 117. 1985. *Roldana sundbergii* Funston, Novon 11: 305. 2001, isonym. TYPE: Mexico. Nuevo León: mpio. Galeana, ca. 25 km S of Iturbide, along main rd. to Agua Blanca, 10.6 km W of Ejido la Purisima, ca. 2100 m, 27 Oct. 1982, *S. Sundberg 1921* (holotype, TEX not seen, TEX digital image TEX!; isotypes, KSC!, NY!).

Perennial herbs, acaulescent, 20–50 cm tall, arising from a fibrous rooted caudex; plants tomentose, tawny at base, floccose to glabrate above; stems terete, striated, reddish. *Leaves* clustered at the base; petioles 2–8 cm, floccose to tawny lanate; blades 6–20 × 5–20 cm, triple-nerved, ovate to oval, 6- to 13-lobed, crenate or weakly secondarily lobed, sinus depth less than 1/4 of the way to the midrib, margins callous denticulate; abaxially lanate tomentose and canescent, adaxially hirsute with multicelled hairs. *Capitulescence* terminal paniculate cyme, surmounting a 4–30 cm naked scape, 3 to 6 capitula per branch (up to 50 capitula in the overall capitulescence), scape glabrate to lanate, primary bracts linear, up to 5 mm, ultimate peduncles 2–10 mm, bracteoles 0 to 2, filiform 1–3 mm. *Capitula* radiate, 5–9 × 2–3 mm, turbinate, corollas yellow; calyculate bracts ca. 2, filiform, 2–3 mm; phyllaries ca. 8, glabrous, greenish, 5–6 × ca. 1 mm. Ray florets 3 to 5, corolla ligulate, glabrous, tube 4–4.5 mm, ligule 6–10 × 2–3 mm. Disk florets 10 to 25, corolla funnelform, glabrous, 5–7 mm, tube 2.5–3 mm, throat ca. 1.5 mm, lobes 2–2.8 mm. Achenes glabrous or sparsely hispid distally, cylindrical, ca. 2 mm, ribs 10, resin glands absent; pappus bristles 5–6 mm.

Distribution and phenology. Mexico. Restricted to the Sierra Madre Oriental of Nuevo León at elevations of 800–2100 m. Found in pine-oak forests with *Pinus*, *Quercus*, *Platanus*, *Populus*, and *Colubrina*. Collected in flower in October.

Comments. *Roldana sundbergii* is perhaps the smallest perennial herb in the genus. It is very similar to *R. gonzaleziae*, but differs in pubescence, leaf shape, and scape size. For a complete review, see the Comments section under *R. gonzaleziae*.

Representative specimens examined. MEXICO. **Nuevo León:** areas cercanas a Cola de Caballo, 100 10′W, 25 23′N, 27 Oct. 1982, *J. A. Villarreal V-4204* (KSC); areas cercanas a Cola de Caballo, bosque mesofilo de montaña, 100 10′W, 24 23′N, 24 Oct. 1987, *J. A. Villarreal 5546* (MO).

48. Roldana tlacotepecana Funston, Novon 11: 305. 2001. TYPE: Mexico. Guerrero: mpio. Tlacotepec, 19.5 km al NE de Puerto del Gallo, camino Atoyac-Filo de Caballo, 2900 m, 23 Nov. 1983, *E. Martínez S. 5648* (holotype, KSC!).

Perennial herbs, acaulescent, 20–60 cm tall, arising from a rhizomatous caudex; plants glabrate to floccose arachnoid, long multicelled hairs present; stems terete, red. *Leaves* clustered at base; petioles 1.5–7 cm, glabrate to weakly arachnoid; blades 3.5–7 × 4–7 cm, triple-nerved, ovate to orbicular, ca. 5-lobed, sinus depth about 1/8 of the way to the midrib, lobes acute or rounded, margin callous denticulate; abaxially lanate tomentose and canescent, adaxially glabrous. *Capitulescence* simple paniculate cyme, borne atop a naked scape, ca. 10 cm, 1 to 2 capitula per branch, pubescence patches of strigose hairs, dense beneath capitula, primary bracts linear, ca. 10 mm, ultimate peduncles when present ca. 4.5 cm, bracteoles absent. *Capitula* radiate, ca. 16 × 7 mm, campanulate, corollas yellow; calyculate bracts ca. 3, linear, 2–4 mm; phyllaries ca. 14, glabrous, ca. 13 × 2 mm. Ray florets ca. 8, corolla ligulate, glabrous, tube ca. 8 mm, ligule ca. 12 × 4 mm. Disk florets ca. 35, corolla funnelform, glabrous, ca. 11 mm, tube ca. 6 mm, throat ca. 4 mm, lobes ca. 1 mm. Achenes glabrous, columnar, ca. 3 mm, ribs 10, resin glands absent; pappus bristles 7–11 mm.

Distribution and phenology. Mexico (Guerrero) at elevations of 2600–3500 m. Found in pine-oak and fir forests. Flowering November through January.

Comments. *Roldana tlacotepecana* is a small herbaceous perennial. It has the pubescence and leaf characteristics of *R. sundbergii* and *R. gonzaleziae* but with larger capitula. It is named after the municipality from which the only known specimens were collected.

Representative specimens examined. MEXICO. **Guerrero:** Cerro Teotepec, mpio. Tlacotepec, ca. 40 km N of Coyuca de Benítez, 3200 m, 12 May 1963, *Feddema 2918* (MICH); Cerro Teotepec, mpio. Tlacotepec, ca. 17°29′N, 100° 12′W, 3400 m, 29 Jan. 1965, *J. Rzedowski 207* (MICH).

Excluded Taxa

Psacaliopsis pinetorum (Hemsl.) Funston & Villaseñor, comb. nov. Basionym: *Senecio pinetorum* Hemsl., Biol. Cent.-Amer., Bot. 2: 245. 1881. *Roldana pinetorum* (Hemsl.) H. Rob. & Brettell, Phytologia 27: 423. 1974. TYPE: Mexico. Oaxaca: Cordillera, 5000–7000 ft., Nov.–Apr. 1840, *Galeotti 2019* (lectotype, designated here,

K not seen; isotypes, G not seen, K fragm. & drawing GH!, G photos F!, GH!, MICH!, MO!).

Distribution and ecology. Mexico, Honduras, and El Salvador (El Salvador: Santa Ana; Honduras: Lempira; Mexico: Guerrero, Oaxaca) at elevations of 2600–3500 m. Found in pine-oak and fir forests. Flowering November through April.

Comments. In the protologue for *Senecio pinetorum*, Hemsley (1881) listed two syntypes, housed at Kew, both from southern Mexico, *Galeotti 2019* and, without locality, *Sallé s.n.* Having only seen the Galeotti gathering, I have chosen it for lectotypification.

The species *Senecio pinetorum* is transferred to the genus *Psacaliopsis* based on the following characters: centrally peltate leaf attachment, orbicular blade shape, and perennial subscapose habit.

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APPENDIX 1. Distribution of species in physiographic regions of Mexico and Central America. Species marked with an asterisk (*) are endemic to that region.

Sierra Madre Occidental (SMO, 11 spp.)

Roldana barba-johannis, *R. cordovensis*, *R. gentryi**, *R. gonzaleziae*, *R. guadalajarensis*, *R. hartwegii*, *R. heracleifolia*, *R. mezquitalana**, *R. sessilifolia*, *R. subpeltata**, *R. suffulta*

Sierras y Llanuras del Norte (SLN, 1 sp.)

R. sessilifolia

Sierra Madre Oriental (SMR, 18 spp.)

R. albonervia, *R. angulifolia*, *R. aschenborniana*, *R. barba-johannis*, *R. grimesii*, *R. hartwegii*, *R. heracleifolia*, *R. hirsuticaulis*, *R. lineolata*, *R. lobata*, *R. metepeca*, *R. neogibsonii**, *R. petasitis*, *R. platanifolia*, *R. reticulata*, *R. reglensis**, *R. sessilifolia*, *R. sundbergii**

Mesa del Centro (MC, 8 spp.)

R. angulifolia, *R. aschenborniana*, *R. cordovensis*, *R. heracleifolia*, *R. hirsuticaulis*, *R. lineolata*, *R. reticulata*, *R. sessilifolia*

Trans-Mexican Volcanic Belt (TVB, 27 spp.)

R. albonervia, *R. aliena**, *R. angulifolia*, *R. aschenborniana*, *R. barba-johannis*, *R. cordovensis*, *R. ehrenbergiana**, *R. glinophylla**, *R. gonzaleziae*, *R. grimesii*, *R. guadalajarensis*, *R. hartwegii*, *R. heracleifolia*, *R. hintonii**, *R. kerberi*, *R. lanicaulis*, *R. lineolata*, *R. lobata*, *R. mexicana*, *R. michoacana**, *R. petasitis*, *R. platanifolia*, *R. reticulata*, *R. robinsoniana*, *R. schaffneri*, *R. sessilifolia*, *R. suffulta*

Sierra Madre del Sur (SMS, 27 spp.)

R. angulifolia, *R. anisophylla**, *R. aschenborniana*, *R. barba-johannis*, *R. cordovensis*, *R. eriophylla*, *R. galiciana*, *R. hartwegii*, *R. hederifolia**, *R. heterogama*, *R. heteroidea**, *R. jurgensenii*, *R. kerberi*, *R. langlassei**, *R. lanicaulis*, *R. lineolata*, *R. lobata*, *R. metepeca*, *R. mexicana*, *R. mixtecana**, *R. petasitis*, *R. platanifolia*, *R. reticulata*, *R. robinsoniana*, *R. schaffneri*, *R. suffulta*, *R. tlacotepeca**

Llanura Costera del Golfo del Sur (LCG, 1 sp.)

R. schaffneri

Sierras de Chiapas y Guatemala (SC & GU, 8 spp.)

R. acutangula, *R. barba-johannis*, *R. greenmanii*, *R. hartwegii*, *R. heterogama*, *R. jurgensenii*, *R. petasitis*, *R. schaffneri*

Cordillera Centroamericana in Mexico (CCM, 8 spp.)

R. acutangula, *R. eriophylla*, *R. gilgii*, *R. heterogama*, *R. jurgensenii*, *R. lanicaulis*, *R. petasitis*, *R. schaffneri*

Central America (CA, 11 spp.)

R. acutangula, *R. aschenborniana*, *R. barba-johannis*, *R. gilgii*, *R. greenmanii*, *R. heterogama*, *R. jurgensenii*, *R. lanicaulis*, *R. petasitis*, *R. scandens**, *R. schaffneri*

APPENDIX 2. Species distribution in Central America.

Guatemala

Roldana acutangula, *R. aschenborniana*, *R. barba-johannis*, *R. giligii*, *R. greenmanii*, *R. heterogama*, *R. jurgensenii*, *R. lanicaulis*, *R. petasitis*, *R. schaffneri*

El Salvador

R. jurgensenii, *R. petasitis*, *R. schaffneri*

Honduras

R. jurgensenii, *R. petasitis*, *R. schaffnerii*

Nicaragua

R. petasitis, *R. schaffneri*

Costa Rica

R. heterogama, *R. scandens*

Panama

R. heterogama

APPENDIX 3. Hypothesized species groups. Physiographic areas from north to south: SMO, Sierra Madre Occidental; SLN, Sierras y Llanuras del Norte; SMR, Sierra Madre Oriental; MC, Mesa del Centro; TVB, Trans-Mexican Volcanic Belt; SMS, Sierra Madre del Sur; LCG, Llanura Costera del Golfo del Sur; SC & GU, Sierras de Chiapas y Guatemala; CCM, Cordillera Centroamericana in Mexico; CA, Central America.

Central American Complex

Roldana acutangula (SC & GU, CCM, CA), *R. gilgii* (CCM, CA), *R. greenmanii* (SC & GU, CA), *R. heterogama* (SMS, SC & GU, CCM, CA), *R. hintonii* (TVB), *R. jurgensenii* (SMS, SC & GU, CCM, CA), *R. mezquitalana* (SMO), *R. scandens* (CA), *R. schaffneri* (TVB, SMS, LCG, SC & GU, CA)

***R. cordovensis* Complex**

R. aliena (SMO, MC, TVB, SMS), *R. angulifolia* (SMR, MC, TVB, SMS), *R. anisophylla* (SMS), *R. cordovensis* (SMO, MC, TVB, SMS), *R. ehrenbergiana* (TVB), *R. gentryi* (SMO), *R. grimesii* (SMR, TVB), *R. hederifolia* (SMS), *R. metepeca* (SMR, SMS), *R. mixtecana* (SMS), *R. platanifolia* (SMR, TVB, SMS)

***R. hartwegii* Complex**

R. hartwegii (SMO, SMR, TVB, SMS, SC & GU), *R. heracleifolia* (SMO, SMR, MC, TVB), *R. kerberi* (TVB, SMS), *R. langlassei* (SMS), *R. lineolata* (SMR, MC, TVB, SMS), *R. tlacotepeca* (SMS)

***R. lobata* Complex**

R. albonervia (SMR, TVB), *R. aschenborniana* (SMR, MC, TVB, CA), *R. barba-johannis* (SMO, SMR, TVB, SMS, SC & GU, CA), *R. eriophylla* (SMS, CCM), *R. gonzaleziae* (SMO, TVB), *R. hirsuticaulis* (SMR, MC), *R. lanicaulis* (TVB, SMS, CCM, CA), *R. lobata* (SMR, TVB, SMS), *R. neogibsonii* (SMR), *R. robinsoniana* (TVB, SMS), *R. sundbergii* (SMR)

***R. pericalia* Complex**

R. glinophylla (TVB), *R. guadalajarensis* (SMO, TVB), *R. heteroidea* (SMS), *R. mexicana* (TVB, SMS), *R. michoacana* (TVB), *R. reglensis* (SMR), *R. reticulata* (SMR, TVB, SMS), *R. sessilifolia* (SMO, SLN, SMR, MC, TVB), *R. subpeltata* (SMO), *R. suffulta* (SMO, TVB, SMS)

***R. petasitis* Complex**

R. petasitis (SMR, TVB, SMS, SC & GU, CCM, CA)

REVISIÓN DEL GÉNERO *JUNELLIA* (VERBENACEAE)[1, 2]

Paola Peralta,[3] *María E. Múlgura de Romero,*[3] *Silvia S. Denham,*[3] *y Silvia M. Botta*[†]

Resumen

Se presenta una revisión del género *Junellia* Moldenke, nom. cons. El género comprende 39 especies y seis variedades distribuidas en América del Sur, desde Perú y Bolivia hasta la Argentina y Chile. Se establece un reordenamiento de las categorías infragenéricas a nivel de secciones; se cambia de subgénero *Junellia* subgén. *Thryothamnus* (Phil.) Botta secc. *Junelliopsis* Botta, dándose la siguiente nueva combinación: *Junellia* subgén. *Junellia* secc. *Junelliopsis* (Botta) P. Peralta & Múlgura; y se funda una nueva sección para las especies excluidas de *Junellia* subgén. *Thryothamnus* secc. *Junelliopsis*: *Junellia* subgén. *Thryothamnus* secc. *Dentium* P. Peralta & Múlgura. Las siguientes secciones y series se sinonimizan: *Junellia* subgén. *Thryothamnus* secc. *Junelliopsis* Botta, *Verbena* L. secc. *Junellia* (Moldenke) Tronc. ser. *Minutifoliae* Tronc., *Verbena* secc. *Junellia* ser. *Thymifoliae* Tronc., *Verbena* secc. *Verbenaca* Walp. ser. *Erinaceae* Walp., *Verbena* secc. *Verbenaca* ser. *Seriphioideae* Walp. y *Verbena* ser. *Pauciflorae* Briq. Se neotipifican: *Verbena juniperina* Lag., *V. ligustrina* Lag., *V. minima* Meyen, *V. thymifolia* Lag., *V. tridactylites* Lag. y *V. tridens* Lag. Se lectotipifican: *Diostea filifolia* Miers, *V. asparagoides* Gillies & Hook. ex Hook., *V. bisulcata* Hayek, *V. connatibracteata* Kuntze, *V. digitata* Phil., *V. lorentzii* Niederl. ex Hieron., *V. ourostachya* Briq., *V. pygmaea* R. E. Fr., *V. selaginoides* Kunth ex Walp., *V. spathulata* Gillies & Hook. ex Hook. var. *grandiflora* Schauer, *V. spathulata* var. *parviflora* Schauer y *V. uniflora* Phil. var. *glabriuscula* Kuntze. Se transfieren a la sinonimia los siguientes taxones: *Junellia echegarayi* (Hieron.) Moldenke var. *cordifolia* Moldenke, *J. echegarayi* var. *puberulenta* Moldenke [= *J. echegarayi*], *J. spathulata* (Gillies & Hook. ex Hook.) Moldenke var. *grandiflora* (Schauer) Botta [= *J. spathulata* var. *glauca* (Gillies & Hook. ex Hook.) Botta], *J. tripartita* Moldenke, *J. longidentata* Moldenke var. *glandulosa* Botta [= *J. aspera* (Gillies & Hook. ex Hook.) Moldenke var. *aspera*], *V. comberi* Sandwith [= *J. caespitosa* (Gillies & Hook. ex Hook.) Moldenke], *V. dolichothyrsa* Sandwith [= *J. connatibracteata* (Kuntze) Moldenke], *V. colchaguensis* Phil. [= *J. lavandulaefolia* (Phil.) Moldenke] y *V. scoparia* Gillies & Hook. ex Hook. var. *puberula* Tronc. [= *J. scoparia* (Gillies & Hook. ex Hook.) Botta]. Se realizan dos nuevas combinaciones a nivel subespecífico: *J. toninii* (Kuntze) Moldenke var. *mulinoides* (Speg.) P. Peralta & Múlgura, *J. aspera* var. *longidentata* (Moldenke) P. Peralta & Múlgura. Se incluye una clave para identificar las especies y variedades, se actualiza la sinonimia de cada una de ellas, que se describen e ilustran y se brinda su distribución geográfica.

Abstract

A systematic treatment of the genus *Junellia* Moldenke, nom. cons. is presented. The genus comprises 39 species and six varieties growing in South America from Peru and Bolivia to Argentina and Chile. Infrageneric names are re-ordered at the sectional level: *Junellia* subg. *Thryothamnus* (Phil.) Botta sect. *Junelliopsis* Botta is moved to another subgenus and the resulting new combination is made: *Junellia* subg. *Junellia* sect. *Junelliopsis* (Botta) P. Peralta & Múlgura; and a new name for taxa excluded from *Junellia* subg. *Thryothamnus* sect. *Junelliopsis* is established: *Junellia* subg. *Thryothamnus* sect. *Dentium* P. Peralta & Múlgura. The following sections and series are synonymized: *Junellia* subg. *Thryothamnus* sect. *Junelliopsis* Botta, *Verbena* L. sect. *Junellia* (Moldenke) Tronc. ser. *Minutifoliae* Tronc., *Verbena* sect. *Junellia* ser. *Thymifoliae* Tronc., *Verbena* sect. *Verbenaca* Walp. ser. *Erinaceae* Walp., *Verbena* sect. *Verbenaca* ser. *Seriphioideae* Walp., and *Verbena* ser. *Pauciflorae* Briq. The following taxa are neotypified: *V. juniperina* Lag., *V. ligustrina* Lag., *V. minima* Meyen, *V. thymifolia* Lag., *V. tridactylites* Lag., and *V. tridens* Lag. Lectotypes for the following species are given: *Diostea filifolia* Miers, *V. asparagoides* Gillies & Hook. ex Hook., *V. bisulcata* Hayek, *V. connatibracteata* Kuntze, *V. digitata* Phil., *V. lorentzii* Niederl. ex Hieron., *V. ourostachya* Briq., *V. pygmaea* R. E. Fr., *V. selaginoides* Kunth ex Walp., *V. spathulata* Gillies & Hook. ex Hook. var. *grandiflora* Schauer, *V. spathulata* var. *parviflora* Schauer, and *V. uniflora* Phil. var. *glabriuscula* Kuntze. The following taxa are synonymized: *Junellia echegarayi* (Hieron.) Moldenke var. *cordifolia* Moldenke, *J. echegarayi* var. *puberulenta* Moldenke [= *J. echegarayi*], *J. spathulata* (Gillies & Hook. ex Hook.) Moldenke var. *grandiflora* (Schauer) Botta [= *J. spathulata* var. *glauca* (Gillies & Hook. ex Hook.) Botta], *J. tripartita* Moldenke, *J. longidentata* Moldenke var. *glandulosa* Botta [= *J. aspera* (Gillies & Hook. ex Hook.) Moldenke var. *aspera*], *V. comberi* Sandwith [= *J. caespitosa* (Gillies & Hook. ex Hook.) Moldenke], *V. dolichothyrsa* Sandwith [= *J. connatibracteata* (Kuntze) Moldenke], *V. colchaguensis* Phil. [= *J. lavandulaefolia* (Phil.) Moldenke], and *V. scoparia* Gillies & Hook. ex Hook. var. *puberula* Tronc. [= *J. scoparia* (Gillies & Hook. ex Hook.) Botta]. Two new combinations of subspecific names are established: *J. toninii* (Kuntze) Moldenke var. *mulinoides* (Speg.) P. Peralta & Múlgura and *J. aspera* var. *longidentata*

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(Moldenke) P. Peralta & Múlgura. A key to identify these species is given, as well as synonymy, descriptions, illustrations, and geographical distribution.

Key words: *Junellia*, Verbenaceae, Verbeneae.

Junellia Moldenke es un género americano representado por 39 especies distribuidas desde Perú y Bolivia hasta la Argentina y Chile.

En 1989, Botta resume la historia taxonómica del género y explica los caracteres usados para la delimitación del mismo dentro de la tribu Verbeneae. Propone además una clasificación infragenérica basada en caracteres morfológicos y anatómicos de órganos vegetativos y reproductivos. Las especies del género *Junellia* no han sido revisadas en su totalidad y sólo han sido tratadas parcialmente en estudios florísticos regionales (Botta, 1984, 1987, 1993, 1999). Dicha autora estaba realizando una revisión integral del género cuando falleció en 1994. En esta contribución se continúa con esta tarea y se revisan las 39 especies del género *Junellia* y sus variedades, sobre la base del estudio de los caracteres morfológicos vegetativos y reproductivos.

Botta (1989: 382) hace referencia a una propuesta de conservación del nombre *Junellia*, pero ésta no había sido aún presentada. Finalmente, Botta et al. (1995) proponen formalmente la conservación del nombre *Junellia* en contra de *Monopyrena* Speg. y *Thryothamnus* Phil. La misma fue recomendada por Brummit (1998: 867), aceptada en el XVII Congreso Internacional de Botánica de Missouri y publicada en el *Código Internacional de Nomenclatura Botánica* (Greuter et al., 2000). Al aceptarse el nombre *Junellia* como nombre conservado y *Monopyrena* y *Thryothamnus* como nombres rechazados, quedó establecida como especie tipo del género *M. serpyllifolia* Speg. [= *J. serpyllifolia* (Speg.) Moldenke] y por lo tanto se hace necesario reordenar los nombres de las divisiones infragenéricas de acuerdo con el *Código Internacional de Nomenclatura Botánica*.

Materiales y Métodos

Los ejemplares examinados provienen del material depositado en los siguientes herbarios: BA, BAA, BAB, CONC, CTES, F, G, H, K, LIL, LP, MERL, MO, NY, P, SI, TEX (Holmgren et al., 1990). Se proveen las listas de las especies aceptadas del género *Junellia* y de los ejemplares examinados en los Apéndices 1 y 2.

Lectotipificaciones. Las especies descritas por Lagasca, en base a las colecciones de L. Née, como integrante de la Expedición Malaspina, fueron destruidos (Fernández Casas & Gamarra, 1993; com. pers. P. Blanco), por eso para las especies de este autor se hizo necesario designar neotipos para las mismas.

Acevedo de Vargas (1951), realizó un estudio crítico de las Verbenáceas de Chile, lectotipificando, en los casos necesarios, los nombres dados por Philippi. Para esto, la autora, utilizó los términos "Typus" e "Isotypus", se considera que los ejemplares señalados por ella como "Typus", deben ser tratados como lectotipos.

Caracteres Morfológicos

Hábito. Las formas biológicas que se observan en el género *Junellia* son nanofanerófitos y caméfitos. Dentro del primer grupo pueden encontrarse: arbustos subáfilos, con hojas usualmente caedizas y generalmente sin desarrollo de braquiblastos (*J. spathulata* (Gillies & Hook. ex Hook.) Moldenke), o arbustos con hojas de los macroblastos y braquiblastos de desarrollo normal o transformadas en espinas (*J. aspa-ragoides* (Gillies & Hook. ex Hook.) Moldenke, *J. aspera* (Gillies & Hook. ex Hook.) Moldenke, *J. seriphioides* (Gillies & Hook. ex Hook.) Moldenke). Los caméfitos pueden ser pulvinados: vegetales leñosos o subleñosos, bajos, con ramas muy apretadas formando cojines hemisféricos más o menos densos; estos cojines en algunos casos son espinosos, densos como en *J. erinacea* (Gillies & Hook. ex Hook.) Moldenke y *J. ulicina* (Phil.) Moldenke, o bien inermes, generalmente con hojas carnosas, laxos como en *J. thymifolia* (Lag.) Moldenke, *J. tridactylites* (Lag.) Moldenke o en placas (con renuevos apenas sobre el nivel del suelo formando placas leñosas de apenas unos centímetros de altura como en *J. aretioides* (R. E. Fr.) Moldenke, *J. micrantha* (Phil.) Moldenke, *J. minima* (Meyen) Moldenke, y *J. uniflora* (Phil.) Moldenke).

Indumento. Los tricomas observados son de dos tipos: glandulares y no glandulares. Los primeros son unicelulares o multicelulares formados por una cabeza globosa (2–8-celular), una célula del cuello y la célula del pie más o menos desarrollada dando origen a pelos glandulares largos y cortos. Los pelos no glandulares presentan un pie o base multicelular y un cuerpo apical unicelular, generalmente con cutícula gruesa. El tipo de pilosidad: híspido, estrigoso, escabroso, etc., y la combinación de los distintos tipos de pelos es constante en las especies estudiadas. Por otra

parte, dentro de una misma especie, la densidad del indumento puede ser muy variable.

Hojas. Las hojas de los macroblastos son opuestas, o a veces tetrásticas, sésiles o brevemente pecioladas, con lámina entera, 2–3-lobada, 2–3-partida o 2–3-secta, de consistencia herbácea, cartácea o crasa, a veces con ápice punzante o bien pueden presentarse reducidas (*Junellia spathulata*) o transformada en espinas (*J. seriphioides*). Las hojas de los braquiblastos en general son dísticas o imbricadas, sésiles, carnosas o no y a veces también transformadas en espinas (*J. ulicina*). La venación es pinnada, con vena primaria única de recorrido recto. En el caso de especies con lámina desarrollada se presentan cinco o seis venas de 2° orden, que pueden presentar vena marginal (*J. longidentata* Moldenke). En especies con hojas de los macroblastos tripartidas y espinescentes, la vena primaria origina dos ramas laterales que ingresan a cada lóbulo, las secundarias son delgadas y puede presentarse vena marginal (*J. tridens* (Lag.) Moldenke).

Inflorescencias. Las inflorescencias son dibotrios heterotéticos o monobotrios, formados por florescencias en espigas contraídas, cilíndricas o corimbiformes, plurifloras o paucifloras, en varias especies reducidas a 2–3 flores (Martínez et al., 1996).

Flor. Las flores son hermafroditas, levemente zigomorfas, con pedicelo muy breve. Cáliz tubular, generalmente 5-dentado, dientes pungentes (*Junellia erinacea*, *J. mulinoides* (Speg.) Moldenke) o no (*J. connatibracteata* (Kuntze) Moldenke, *J. uniflora*), en general subiguales, los abaxiales más desarrollados que los adaxiales. La morfología de los dientes calicinales, por lo común, es constante para cada especie. La corola es hipocraterimorfa, conspicua, blanca, blanco-crema, amarillenta, rosa a rojiza o lila, generalmente con tubo bien desarrollado, exteriormente glabro, pubescente o en algunos casos glanduloso (*J. ulicina*), interiormente con una banda longitudinal y abaxial de pelos agudos, adpresos y retrorsos; fauce glabra, a veces con escasos pelos, semejantes a los del interior del tubo corolino, se observaron pelos moniliformes, en *J. tetragonocalyx* (Tronc.) Moldenke. Los estambres son cuatro, didínamos, insertos en la mitad superior del tubo corolino, con filamentos breves; el conectivo puede o no superar la longitud de las tecas, algunas veces se presentan apéndices conectivales desarrollados. El gineceo presenta el estilo filiforme, más de tres veces el largo del ovario, la base del estilo es ensanchada, cubriendo el ápice de las clusas (subgén. *Junellia*) o no desarrollada (subgén. *Thryothamnus*).

Polen. Los granos de polen son radialmente simétricos, isopolares, elípticos en vista ecuatorial, y triangulares en vista polar, tricolpados sin ornamentaciones notables (Raj, 1983).

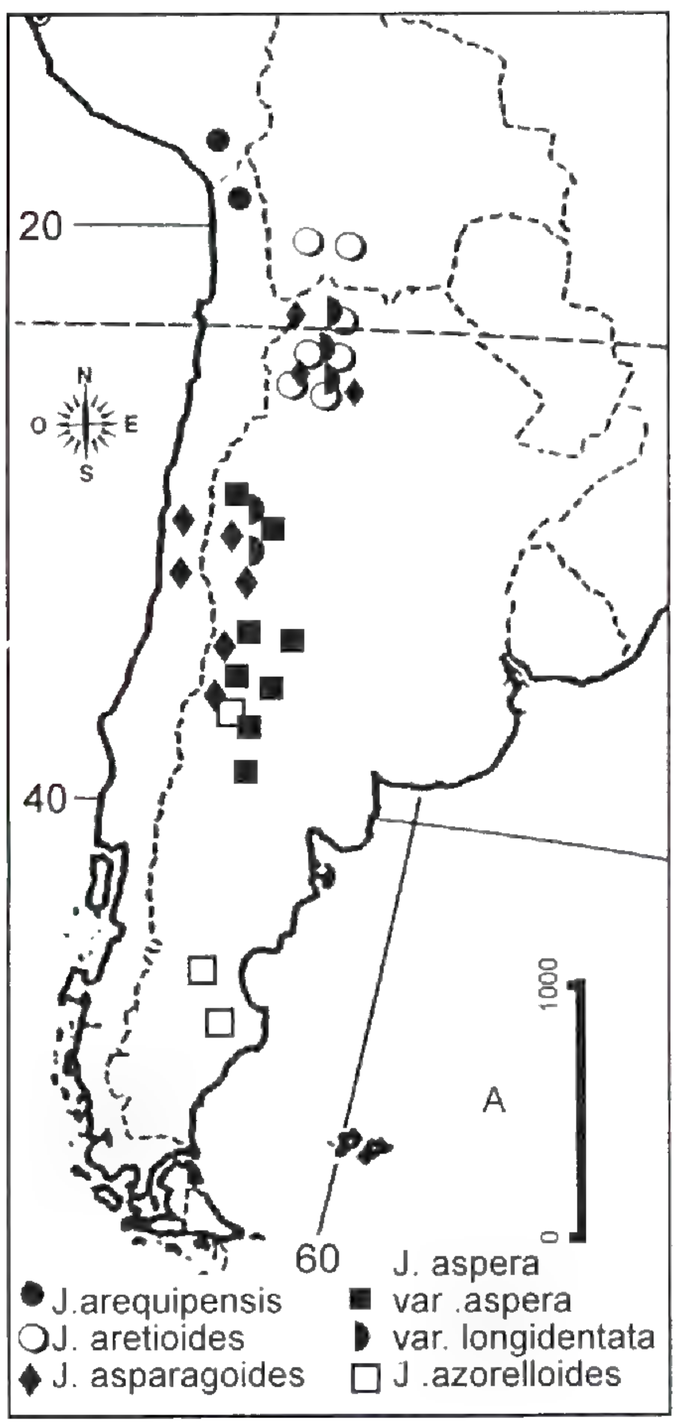
Fruto. Clusas subcilíndricas y de sección subtrígona, a veces con expansiones laterales y laminares del pericarpio que forman dos alas notables (*Junellia* sección *Verticiflorae* sensu Botta), de base angostada y sin desarrollo de un repliegue interno y basal de la cara comisural. El ápice puede ser rostrado como en *J. bisulcata* (Hayek) Moldenke.

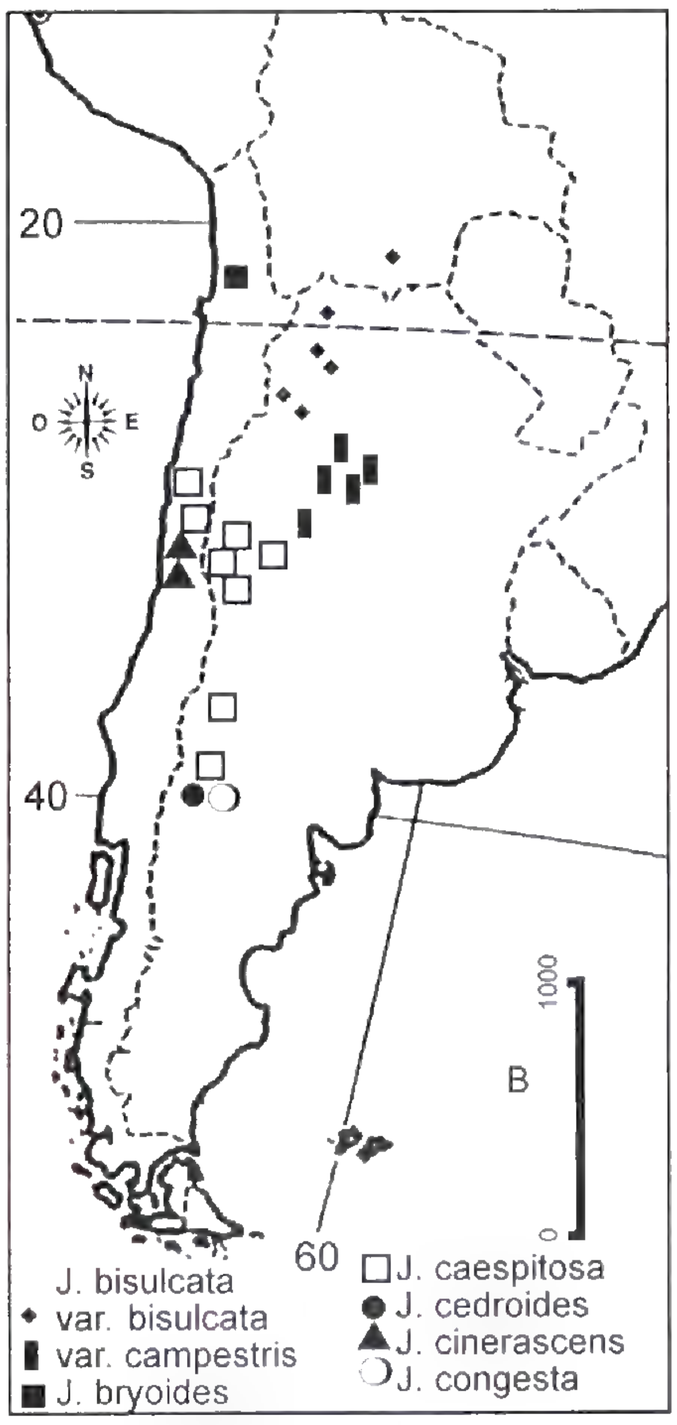
Número cromosómico. De acuerdo con Botta y Brandham (1993) el número cromosómico puede ser $x = 9$ o $x = 10$.

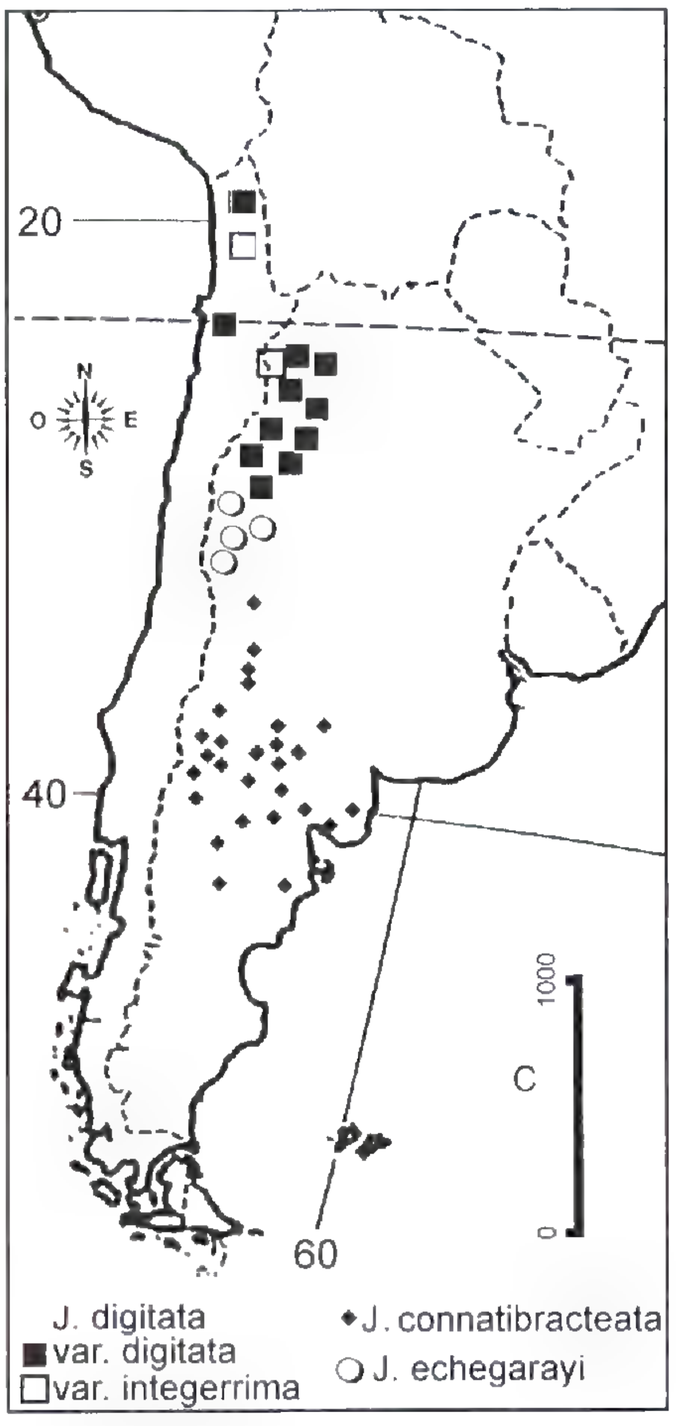
Distribución y Hábitat

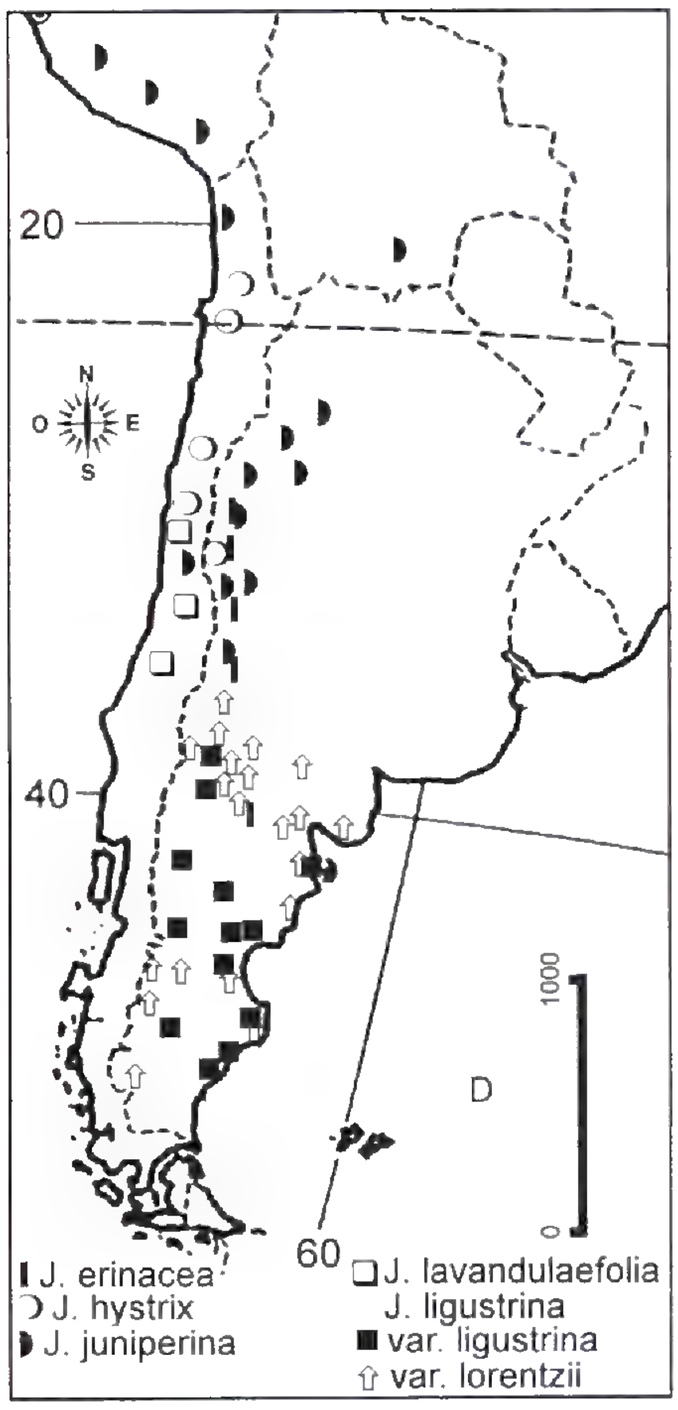
El género *Junellia* se extiende en forma continua desde Perú y Bolivia hasta el sur de la Argentina y Chile, abarcando aproximadamente desde los 65° a los 75° de longitud oeste y desde los 16° a los 51° de latitud sur, creciendo desde el nivel del mar, en la Patagonia argentina, y hasta los 4600 m en la región andina (Figs. 1, 2). El mayor número de especies se encuentra en la Patagonia argentina. Algunas especies son exclusivas de Chile (*J. bryoides* (Phil.) Moldenke, *J. cinerascens* (Schauer) Botta, *J. lavandulaefolia* (Phil.) Moldenke, *J. selaginoides* (Walp.) Moldenke) o Perú (*J. arequipensis* (Botta) Botta).

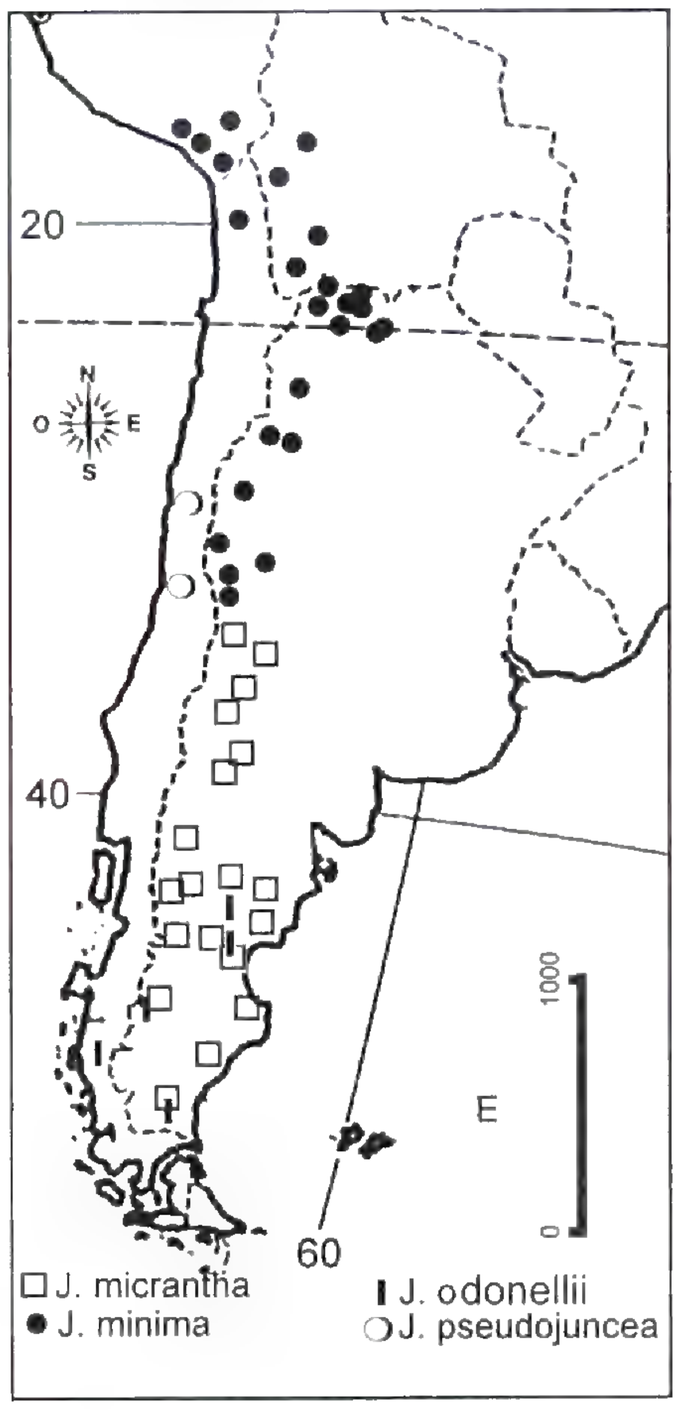
Fitogeográficamente, se observa una mayor diversidad de taxones en el Dominio Andino-patagónico (Cabrera & Willink, 1973). Ciertas especies de *Junellia* son elementos importantes en las comunidades regionales (Boelcke et al., 1985); así, por ejemplo, *J. seriphioides* es frecuente en la provincia Patagónica y en la provincia Puneña (Cabrera, 1957, 1978; Roig, 1970); Morello (1958: 117) la menciona, bajo *Verbena* L. y junto con *J. aspera* y *J. juniperina* (Lag.) Moldenke, como una de las especies importantes en las estepas de arbustos bajos en los faldeos de la provincia del Monte. Otras especies son frecuentes en algunos distritos de la provincia Patagónica: *J. tridens* es muy abundante en la provincia de Santa Cruz, subdistrito santacrucence del Distrito Central (Soriano, 1956a, 1983). *J. ligustrina* (Lag.) Moldenke es frecuente en el Distrito del Golfo de San Jorge y en la parte septentrional del Distrito Central y *J. connatibracteata* y *J. succulentifolia* (Kuntze) Moldenke aparecen en el Distrito Occidental (Cabrera, 1978). Algunos otras especies tienen un área más restringida, por ejemplo *J. congesta* (Tronc.) Moldenke y *J. cedroides* (Sandwith) Moldenke, endémicas de la provincia Patagónica











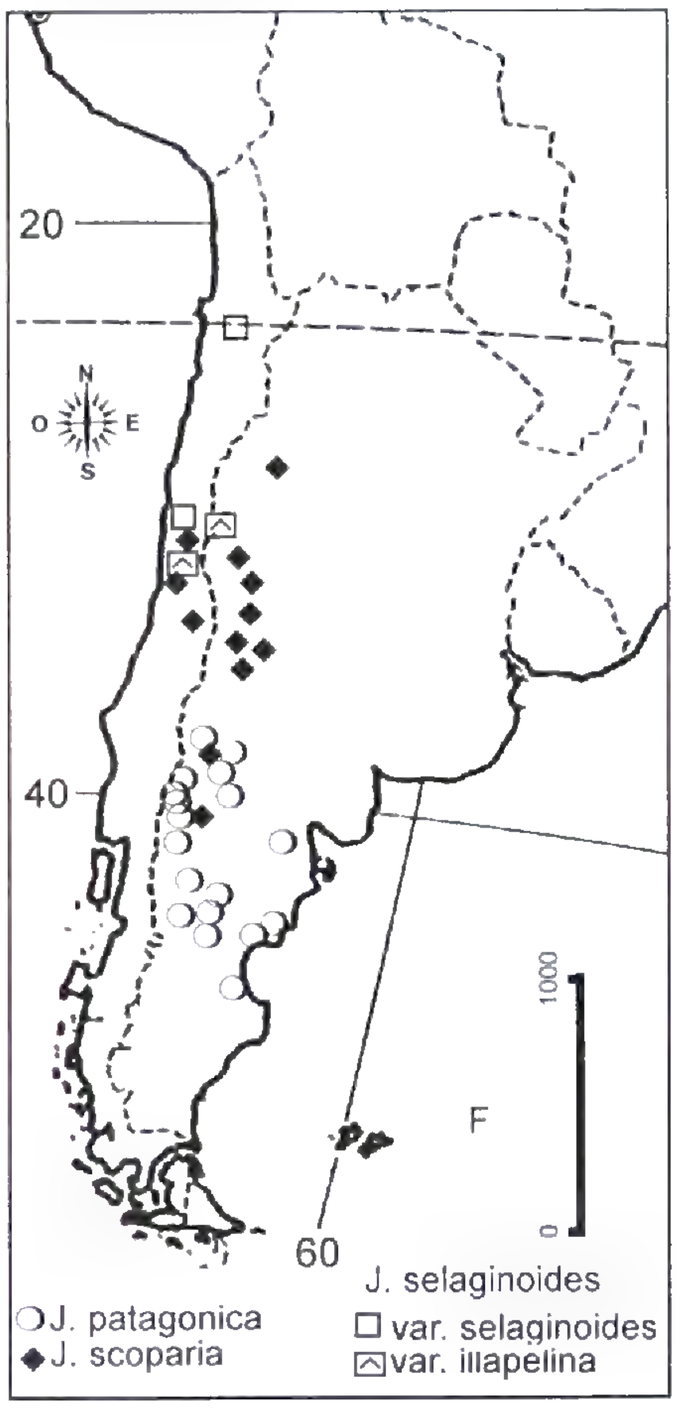


Figura 1. Distribución de: —A. *Junellia arequipensis*, *J. aretioides*, *J. asparagoides*, *J. aspera*, *J. azorelloides*. —B. *J. bisulcata*, *J. bryoides*, *J. caespitosa*, *J. cedroides*, *J. cinerascens*, *J. congesta*. —C. *J. digitata*, *J. connatibracteata*, *J. echegarayi*. —D. *J. erinacea*, *J. hystrix*, *J. juniperina*, *J. lavandulaefolia*, *J. ligustrina*. —E. *J. micrantha*, *J. minima*, *J. odonellii*, *J. pseudojuncea*. —F. *J. patagonica*, *J. scoparia*, *J. selaginoides*.

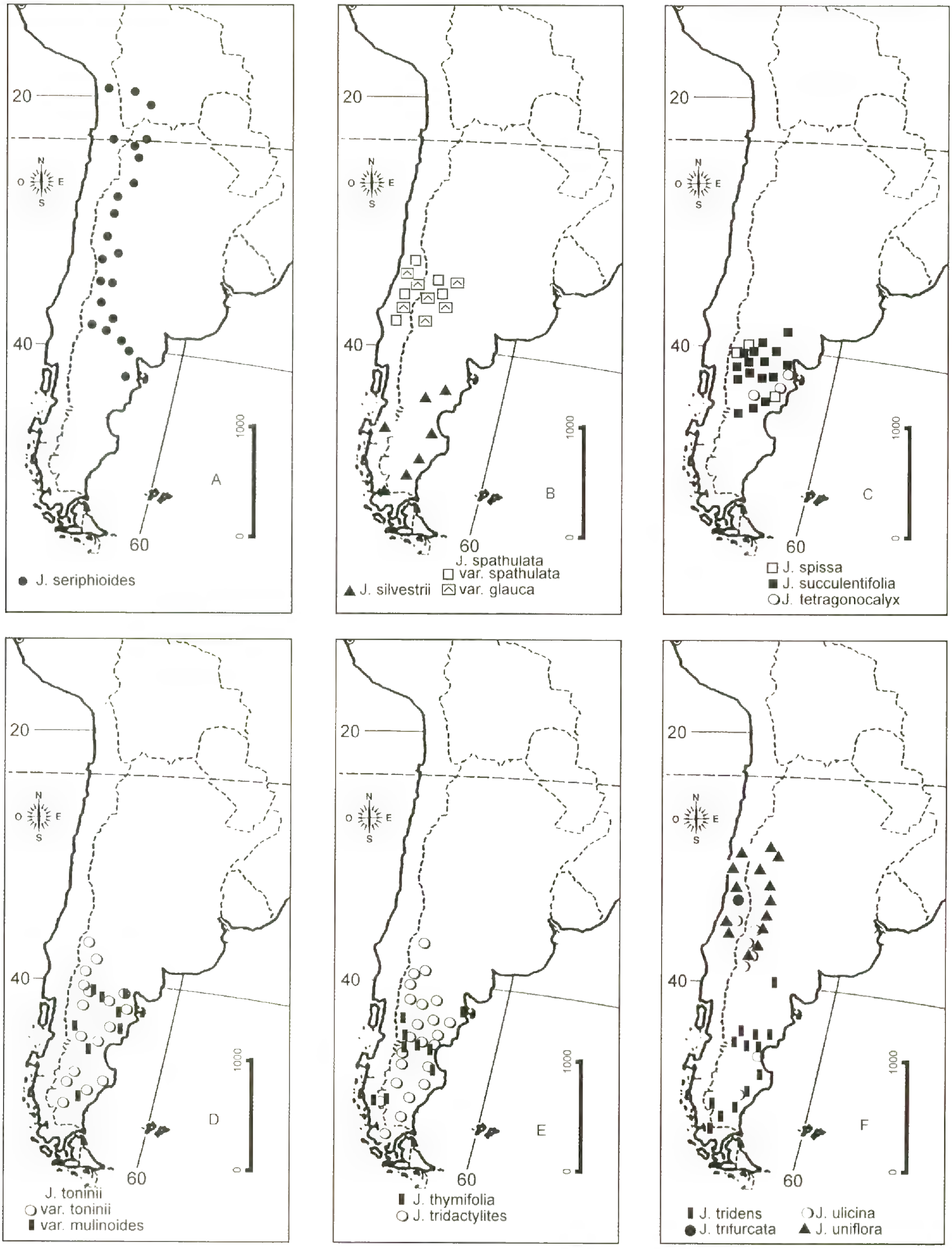


Figura 2. Distribución de: —A. *Junellia seriphioides*. —B. *J. silvestrii*, *J. spathulata*. —C. *J. spissa*, *J. succulentifolia*, *J. tetragonocalyx*. —D. *J. toninii*. —E. *J. thymifolia*, *J. tridactylites*. —F. *J. tridens*, *J. trifurcata*, *J. ulicina*, *J. uniflora*.

argentina, la primera con un área restringida al departamento Zapala, provincia de Neuquén, mientras que la segunda se encuentra en Zapala y en el departamento Pilcaniyeu, provincia de Río Negro.

Tratamiento Taxonómico

Dentro de la familia Verbenaceae, *Junellia* se ubica en la tribu Verbeneae Schauer (Atkins, 2004: 459),

caracterizada por: fruto seco esquizocárpico, separado a la madurez en cuatro clusas; anteras con tecas paralelas, o apenas divergentes, a menudo con conectivo dilatado; estilo 2-lobado, lóbulo anterior estigmatífero.

Clave para Reconocer los Géneros de la Tribu Verbeneae

1a. Estambres fértiles 2; cáliz acrescente en el fruto . . . . . *Hierobotana* Briq.
1b. Estambres fértiles 4, cáliz no acrescente en el fruto.
  2a. Arbustos con hojas bien desarrolladas o subáfilos, espinescente o no, o caméfitos pulvinados o en placas.
    3a. Cáliz con largos pelos higroscópicos que cubren casi por completo a la corola; exocarpo liso . . . . *Urbania* Phil.
    3b. Cáliz sin largos pelos higroscópicos; exocarpo generalmente reticulado . . . . . .*Junellia* Moldenke
  2b. Hierbas anuales o perennes, con hojas bien desarrolladas, no espinescentes.
    4a. Sinflorescencia en dibotrio o pleiobotrio heterotético; espigas cilíndricas laxas densas en la floración . . *Verbena* L.
    4b. Sinflorescencia en monobotrio o pleiobotrio heterotético; espigas generalmente contraídas en la floración . . . . . .*Glandularia* J. F. Gmel.

Junellia Moldenke, Lilloa 5: 392. 1940, nom. cons. TIPO: *Monopyrena serpyllifolia* Speg. [= *Junellia serpyllifolia* (Speg.) Moldenke].

Monopyrena Speg., Rev. Agron. La Plata 3: 559. 1897, nom. rej.

Thryothamnus Phil., Anales Univ. Chile 90: 618. mai 1895, nom. rej. TIPO: *Thryothamnus junciformis* Phil.

Verbena sect. II *Junellia* (Moldenke) Tronc., *Darwiniana* 18: 312. 1974. TIPO: *Verbena erinacea* Gillies & Hook. ex Hook. [= *Junellia erinacea* (Gillies & Hook. ex Hook.) Moldenke].

Arbustos bajos o caméfitos, inermes o no, tallos de sección poligonal o cilíndrica, con desarrollo o no de braquiblastos. Hojas homomorfas o dimorfas, opuestas, a veces imbricadas, sésiles, lámina desarrollada o no, enteras, lobuladas, partidas o sectadas, pungentes o no. Monobotrios o dibotrios, bifloros hasta plurifloros, raquis alargado o no en la fructificación. Flores levemente zigomorfas; cáliz tubuloso, 4- o 5-dentado, diente adaxial breve persistente, no contortos en la fructificación; corola hipocraterimorfa (4–)5-lobada, interior del tubo corolino con pelos retrorsos, fauce glabra o con pelos semejantes o no a los del interior del tubo; estambres 4, didínamos, insertos en la mitad superior del tubo corolino, generalmente incluidos, tecas paralelas, conectivo mayor o menor que las tecas, con o sin apéndice glandular; ovario generalmente glabro (*Junellia tridens* con ápice piloso), 2-carpelar, 4-locular, 4-ovular, óvulos basales; estilo filiforme, más de 3 veces el largo del ovario, con la base ensanchada o no, estigma bilobado, lóbulo anterior estigmatífero. Clusas 4, subtrígonas, margen alado o no, ápice truncado o no, base angostada, sin repliegue interno de la cara comisural, blanca y generalmente papilosa y cara dorsal castaña, generalmente reticulada.

Clave de Subgéneros, Especies y Variedades de *Junellia*

1a. Arbustos bajos o caméfitos; conectivo no superando la longitud de las tecas; base del estilo ensanchada y cubriendo total o parcialmente el ápice de las clusas: monobotrios pauci- o plurifloros (*Junellia* subgén. *Junellia*).
  2a. Hojas generalmente homomorfas, nunca transformadas en espinas, sin ápice punzante; arbustos inermes o caméfitos.
    3a. Par superior de estambres con apéndices conectivales; corola glabra exteriormente; hojas generalmente trilobadas a tripartidas; caméfitos; monobotrios pauci- o plurifloros (*Junellia* secc. *Junellia*) . . . . . .1. *J. micrantha* (Phil.) Moldenke
    3b. Par superior de estambres sin apéndices conectivales; corola con pubescencia variable exteriormente; hojas enteras o trilobadas a tripartidas; arbustos bajos o caméfitos; monobotrios pauci- o plurifloros (*Junellia* secc. *Guedesia*).
      4a. Inflorescencias con 1 a 4 flores; caméfitos.
        5a. Hojas enteras.
          6a. Cáliz glabro; hojas generalmente tetrásticas, glabras o apenas ciliadas en el margen . . . . . 8. *J. silvestrii* (Speg.) Moldenke
          6b. Cáliz con pubescencia variada sobre la superficie; hojas no tetrásticas, pubescentes o hirsutas.
            7a. Cáliz pubérulo en la zona apical; clusas con las caras comisurales papilosas . . . . . 6. *J. minima* (Meyen) Moldenke
            7b. Cáliz pubescente en toda su superficie; clusas generalmente con las caras comisurales lisas . . . . . 13. *J. uniflora* (Phil.) Moldenke
        5b. Hojas tripartidas o trilobadas.
          8a. Hojas tripartidas, lóbulos con ápice agudo y punzante . . 12. *J. trifurcata* (Phil.) Moldenke
          8b. Hojas trilobadas, algunas enteras, lóbulos con ápice redondeado no punzante . . . . . 2. *J. aretioides* (R. E. Fr.) Moldenke
      4b. Inflorescencias con más de 4 flores; caméfitos o arbustos bajos.
        9a. Caméfitos hasta 5 cm alt.; hojas enteras o no.
          10a. Hojas generalmente todas enteras, de margen revoluto.

11a. Hojas lineares, pubérulas; cáliz y tubo corolino hispídulo, éste apenas más largo que el cáliz . . . . . 3. *J. azorelloides* (Speg.) Moldenke
11b. Hojas ovadas, pilosas; cáliz cano-velloso, tubo corolino velloso, éste el doble de largo que el cáliz . . . . . 7. *J. patagonica* (Speg.) Moldenke
10b. Hojas tripartidas o trilobadas.
12a. Hojas glabras, lóbulos enteros; brácteas enteras, oblongas; corola apenas mayor que el cáliz, exteriormente pubescente . . . . . 4. *J. congesta* (Tronc.) Moldenke
12b. Hojas con pubescencia variable.
13a. Hojas híspidas, lóbulos divididos; corola dos veces la longitud del cáliz, exteriormente glabra.
14a. Brácteas tripartidas . . . . . 5a. *J. digitata* var. *digitata* (Phil.) Moldenke
14b. Brácteas enteras, anchamente ovadas . . . . . .5b. *J. digitata* var. *integerrima* (Botta) Botta
13b. Hojas con pubescencia adpresa; lóbulos enteros; corola menos de dos veces la longitud del cáliz, exteriormente con pelos aplicados retrorsos . . . . . 11. *J. tridactylites* (Lag.) Moldenke
9b. Arbustos de desarrollo variable; hojas enteras y algunas trilobadas.
15a. Hojas con pelos cortos simples y glandulares; tallos con pubescencia escabrosa, con pelos glandulares . . . . . 9. *J. succulentifolia* (Kuntze) Moldenke
15b. Hojas con pelos largos blanquecinos, no glandulares; tallos con pelos adpreso-retrorsos, sin pelos glandulares . . . . . 10. *J. thymifolia* (Lag.) Moldenke
2b. Hojas homomorfas, todas espiniformes o dimorfas, las de los macroblastos espiniformes o al menos con ápice punzante, las de los braquiblastos, tetrásticas, carnosas; caméfitos pulvinados o arbustos espinosos (*Junellia* secc. *Junelliopsis*).
16a. Hojas homomorfas, espiniformes, enteras; caméfitos pulvinados; clusas brevemente rostradas . . . . . .18. *J. erinacea* (Gillies & Hook. ex Hook.) Moldenke
16b. Hojas dimorfas; arbustos o caméfitos pulvinados; clusas generalmente truncadas u obtusas, a veces rostradas.
17a. Hojas de los macroblastos la mayoría enteras.
18a. Inflorescencias bifloras (raro 3–4-floras) en los braquiblastos.
19a. Arbustos de 30–80 cm alt.; clusas brevemente aladas . . . . . 21. *J. seriphioides* (Gillies & Hook. ex Hook.) Moldenke
19b. Caméfitos hasta 30 cm alt.; clusas no aladas.
20a. Brácteas híspidas en la cara externa; cáliz 3.5–4 mm; gineceo de 3.5–4.5 mm . . . . . 22. *J. spissa* (Sandwith) Moldenke
20b. Brácteas pubescentes en ambas caras; cáliz de 4.5–5 mm; gineceo de 6.5–7 mm . . . . . 16. *J. caespitosa* (Gillies & Hook. ex Hook.) Moldenke
18b. Inflorescencias 3–4- a plurifloras, en los macroblastos o en braquiblastos alargados; arbustos bajos.
21a. Brácteas connadas, ovadas, pubescentes; cáliz híspido sobre los nervios; hojas recurvas, a veces algunas hojas más o menos rectas . . . . . .17. *J. connatibracteata* (Kuntze) Moldenke
21b. Brácteas no connadas, ovadas o angostamente ovadas, agudas hasta acuminadas, pilosas o glabras; cáliz pubérulo a pubescente o glabro; hojas rectas.
22a. Brácteas pilosas; cáliz con pubescencia diversa: pubérulos hasta pubescentes . . . . . 23a. *J. toninii* (Kuntze) Moldenke var. *toninii*
22b. Brácteas glabras; cáliz glabro, sólo ciliado en los márgenes . . . . . .23b. *J. toninii* var. *mulinoides* (Speg.) P. Peralta & Múlgura
17b. Hojas de los macroblastos tripartidas o trisectas, punzantes, a veces también con hojas enteras, en el mismo ejemplar.
23a. Clusas rostradas; hojas de los braquiblastos tripartidas; brácteas enteras; tallos con pubescencia adpreso-retrorsa; arbustos bajos.
24a. Cáliz con dientes triangulares, menores a 1 mm, pubescencia híspida; hojas de macroblastos generalmente patentes . . . 14a. *J. bisulcata* (Hayek) Moldenke var. *bisulcata*
24b. Cáliz con dientes lineares atenuados, de 1–1.5 mm, pubescencia hirsuta; hojas de los macroblastos no patentes . . . . . 14b. *J. bisulcata* var. *campestris* (Griseb.) Botta
23b. Clusas no rostradas; hojas de braquiblastos enteras o bi- o tripartidas; brácteas enteras o tripartidas; tallo con pubescencia híspida, pelos patentes o glabrescentes; arbustos bajos o caméfitos pulvinados.
25a. Brácteas tripartidas; hojas de los braquiblastos tripartidas.
26a. Monobotrios de 3 a 10 flores; brácteas de 4.5–7 mm; dientes del cáliz de 0.5 mm long., con ápice atenuado . . . . . 20. *J. odonellii* Moldenke
26b. Monobotrios de 2 o 3 flores; brácteas de 11.5–12 mm; dientes del cáliz de 1.5 mm long., con ápice acuminado . . . . . 24. *J. ulicina* (Phil.) Moldenke
25b. Brácteas enteras; hojas de los braquiblastos enteras o tripartidas.
27a. Arbustos con ramas erguidas, hasta de 1 m alt.; hojas de los braquiblastos generalmente enteras o bilobadas o bi- o trisectas, punzantes; tallos con

pubescencia patente . . . . . 19. *J. juniperina* (Lag.) Moldenke

27b. Arbustos postrados o caméfitos; hojas de los braquiblastos todas enteras, no punzantes; tallos con pubescencia patente o glabrescentes.

28a. Brácteas agudas o acuminadas, iguales o menores que la longitud del tubo calicino; hojas, brácteas y cáliz escasamente pilosas o glabras; tallos glabrescentes.

29a. Brácteas pilosas; cáliz con pubescencia diversa: pubérulos hasta pubescentes . . . . . 23a. *J. toninii* var. *toninii*

29b. Brácteas glabras; cáliz glabro, sólo ciliado en los márgenes . . . . . 23b. *J. toninii* var. *mulinoides*

28b. Brácteas ovadas y de ápice agudo, menor que la mitad de la longitud del tubo calicino; hojas, brácteas y cáliz pilosos en toda su superficie; tallos con pelos patentes . . . . . 15. *J. bryoides* (Phil.) Moldenke

1b. Arbustos, no formando caméfitos; conectivo generalmente superando la longitud de las tecas (excepto secc. *Thryothamnus*); base del estilo no ensanchada y más o menos hundida entre las cuatro clusas (*Junellia* subgén. *Thryothamnus*).

30a. Corola de fauce glabra; hojas enteras, lineares (muy rara vez bi- o tetralobadas) no espiniformes, papiráceas o crasas; espigas contraídas; estambres con conectivo menor que las tecas; clusas castañas (*Junellia* secc. *Thryothamnus*).

31a. Arbustos con hojas subopuestas, a veces alternas, y generalmente braquiblastos bien desarrollados.

32a. Cáliz con dientes subulados, en general mayores de 1 mm long., con pubescencia híspida y abundancia de pelos glandulares largos; hojas de 5–15(–17) mm long. . . . . 27a. *J. selaginoides* (Kunth ex Walp.) Moldenke var. *selaginoides*

32b. Cáliz con dientes triangulares y agudos, menores de 1 mm long., con pubescencia híspida y escasos pelos glandulares largos; hojas hasta de 5 mm long. . . . . 27b. *J. selaginoides* var. *illapelina* (Phil.) Botta

31b. Arbustos con hojas opuestas, generalmente sin braquiblastos desarrollados.

33a. Brácteas lineares, ápice agudo, apenas menores que la longitud total del cáliz . . . . . 26. *J. pseudojuncea* (Gay) Moldenke

33b. Brácteas no lineares.

34a. Brácteas angostamente elípticas, ápice acuminado, no punzante . . . . . 25. *J. lavandulaefolia* (Phil.) Moldenke

34b. Brácteas ovadas, generalmente ápice rígido, punzante.

35a. Brácteas todas similares, menores o iguales a la mitad de la longitud del cáliz, de 0.5–0.6 mm lat., margen no membranáceo . . . . . 28a. *J. spathulata* (Gillies & Hook. ex Hook.) Moldenke var. *spathulata*

35b. Brácteas diferentes, siempre mayores a la mitad de la longitud del cáliz: las basales de 3–4 mm lat., margen membranáceo o no, las medias y superiores más angostas, de 2–3.5 mm lat., margen no membranáceo . . . . . 28b. *J. spathulata* var. *glauca* (Gillies & Hook. ex Hook.) Botta

30b. Corola de fauce pubescente o glabra; hojas enteras o tripartidas, inermes o punzantes; monobotrios en espigas contraídas o alargadas; estambres con conectivo mayor que las tecas.

36a. Hojas de los macroblastos espiniformes, las de los braquiblastos con lámina reducida o no; monobotrios en espigas contraídas hasta 3 cm long.; corola con fauce pubescente (*Junellia* secc. *Dentium*).

37a. Hojas de los macroblastos enteras; cáliz 4-dentado, 4-costado; fauce de la corola con pelos moniliformes . . . . . 32. *J. tetragonocalyx* (Tronc.) Moldenke

37b. Hojas de los macroblastos tripartidas; cáliz 5-dentado, 5-costado; fauce de la corola con pelos claviformes.

38a. Gineceo piloso en el ápice . . . . . 33. *J. tridens* (Lag.) Moldenke

38b. Gineceo glabro en el ápice.

39a. Cáliz campanulado con dientes triangulares de ápice agudo . . . . . 30. *J. cedroides* (Sandwith) Moldenke

39b. Cáliz de dientes subulados, punzante.

40a. Hojas de los braquiblastos enteras con lámina desarrollada, ovado- a lineal-oblonga, a veces con un diminuto mucrón . . . . . 29. *J. asparagoides* (Gillies & Hook. ex Hook.) Moldenke

40b. Hojas de los braquiblastos tripartidas, con lámina reducida, carnosa . . . . . 31. *J. hystrix* (Phil.) Moldenke

36b. Hojas de los macroblastos no espiniformes, las de los braquiblastos con lámina desarrollada; monobotrio o dibotrios en espigas mayores de 3 cm long.; corola con fauce glabra (excepto en *J. scoparia* y *J. cinerascens*) (*Junellia* secc. *Verticiflora*).

41a. Plantas generalmente sin desarrollo de braquiblastos; fauce de la corola pubescente; hojas de 2–8(–15) mm de largo, membranáceas o subcrasas.

42a. Tallos de sección tetragonal; apéndices conectivales presentes; base del estilo profundamente hundida entre las clusas; plantas grisáceas que no se ennegrecen al secarse . . . . . 36. *J. cinerascens* (Schauer) Botta

42b. Tallos de sección poligonal; apéndices conectivales ausentes; base del estilo apenas hundido entre las clusas; plantas que se ennegrecen al secarse . . . . . .39. *J. scoparia* (Gillies & Hook. ex Hook.) Botta

41b. Plantas con diferenciación en braquiblastos y macroblastos; fauce de la corola glabra; hojas de los macroblastos de 5–25 mm de long., cartáceas o coriáceas.

43a. Clusas con el pericarpo apenas expandido lateralmente formando un ala insconspicua; dientes del cáliz triangulares, menos de 1 mm de long.; tubo corolino 2–3 veces la longitud del cáliz . . . . . 34. *J. arequipensis* (Botta) Botta

43b. Clusas con el pericarpo expandido lateralmente, formando un ala conspicua.

44a. Hojas coriáceas y enteras; dientes del cáliz triangulares hasta de 1 mm long., pubescencia del cáliz adpresa o híspida; corola más de 2 veces la longitud del cáliz.

45a. Cáliz con pubescencia adpresa; inflorescencia de (3–)4.5–9 cm long. . . . . 38a. *J. ligustrina* var. *ligustrina* (Lag.) Moldenke

45b. Cáliz con pubescencia híspida; inflorescencia de 1.5–3(–6) cm long. . . . . 38b. *J. ligustrina* var. *lorentzii* (Niederl. ex Hieron.) Moldenke

44b. Hojas membranáceas, enteras o trisectas en los macroblastos.

46a. Dientes del cáliz subulados, de 1–2.5 mm long.

47a. Pubescencia del cáliz híspido-glandulosa o escabroso-glandulosa; tubo de la corola de 6.5–10.5 mm, apenas más largo que el cáliz; ramas jóvenes de sección circular; hojas del macroblasto generalmente trisectas . . . . . 35a. *J. aspera* (Gillies & Hook. ex Hook.) Moldenke var. *aspera*

47b. Pubescencia del cáliz incano-vellosa, no glandulosa, más abundante sobre las costillas; tubo de la corola de 8–9.5 mm, generalmente 2 veces el largo del cáliz; ramas jóvenes de sección tetrágona; hojas del macroblasto generalmente enteras . . . . . .35b. *J. aspera* var. *longidentata* (Moldenke) Múlgura & P. Peralta

46b. Dientes del cáliz triangulares, subiguales, de ápice rígido, los abaxiales de 1 mm long.; pubescencia del cáliz homogeneamente híspidula; corola menos de 2 veces la longitud del cáliz . . . . . 37. *J. echegarayi* (Hieron.) Moldenke

Junellia Moldenke subgén. **Junellia**. TIPO: *Monopyrena serpyllifolia* Speg. [= *Junellia serpyllifolia* (Speg.) Moldenke].

Verbena secc. *Verbenaca* Walp., p.p., Repert. Bot. Syst. 4: 14. 1845.

Arbustos bajos, muy ramificados, con láminas de las hojas bien desarrolladas, a veces espiniformes o caméfitos; conectivo no superando la longitud de las tecas; base del estilo ensanchada y cubriendo el ápice de las clusas.

Observaciones. La sección *Verbenaca* Walp. comprendía muchas de las especies que actualmente se consideran pertenecientes a los géneros *Glandularia* y *Verbena*. Schauer (1847: 536) funda la sección *Verbenaca*, que resulta ser un homónimo posterior.

Junellia Moldenke subgén. **Junellia** secc. **Junellia**. TIPO: *Monopyrena serpyllifolia* Speg. [= *Junellia serpyllifolia* (Speg.) Moldenke].

Verbena secc. *Junellia* ser. *Minutifoliae* Tronc., p.p., Darwiniana 18: 314. 1974, syn. nov. *Junellia* secc. II *Guedesia* Botta, ser. *Minutifoliae* (Tronc.) Botta, p.p., Darwiniana 29: 389. 1989.

Caméfitos. Hojas homomorfas, enteras o no, inermes. Monobotrios plurifloros. Apéndices conectivales desarrollados. Número cromosómico $x = 9$, $x = 10$.

Observaciones. Botta (1989: 386), siguiendo el criterio de Troncoso (1974: 312) eligió *Verbena erinacea* Gillies & Hook. ex Hook. como especie tipo del subgénero. Al realizar la propuesta de conservación del nombre (Botta et al., 1995) la especie tipo del género pasó a ser *Junellia serpyllifolia*. La misma había sido considerada por Botta (1989: 390) dentro de la sección *Guedesia* Botta, ser. *Minutifoliae* (Tronc.) Botta. Con posterioridad Botta y Brandham (1993: 150) no la asignan a ninguna de las divisiones infragenéricas propuestas hasta ese momento, por tener un número cromosómico diferente a las restantes especies de la sección *Guedesia*. Asimismo los autores, sugerian la necesidad de la reclasificación de la especie en una nueva serie o sección, criterio que se ha adoptado en este tratamiento.

Esta sección queda conformada por esta única especie por sus características morfológicas y cromosómicas. Las restantes especies de la serie *Minutifoliae* Tronc., permanecen en la sección *Guedesia*.

1. **Junellia micrantha** (Phil.) Moldenke, Lilloa 5: 399. 1940. Basónimo: *Verbena micrantha* Phil., Anales Univ. Chile 90: 915. 1895. TIPO: Chile o Argentina. "Andes provincianus centralis", 1872, *P. Ortega s.n.* (holotipo, BM!; isotipo, BM foto SI!). Figura 3A–E.

Verbena ramulosa Phil., Anales Univ. Chile 90: 610. 1895. TIPO: Chile. "Prope locum Angostura de Peine in provincia O'Higgins lecta est", 1 nov. 1886, *J. Monreal Marin s.n.* (holotipo, SGO!, SGO foto SI!; isotipo, SI!).

Monopyrena serpyllifolia Speg., Revista Fac. Agron. Univ. Nac. La Plata 3: 559. 1897. *Verbena serpyllifolia*

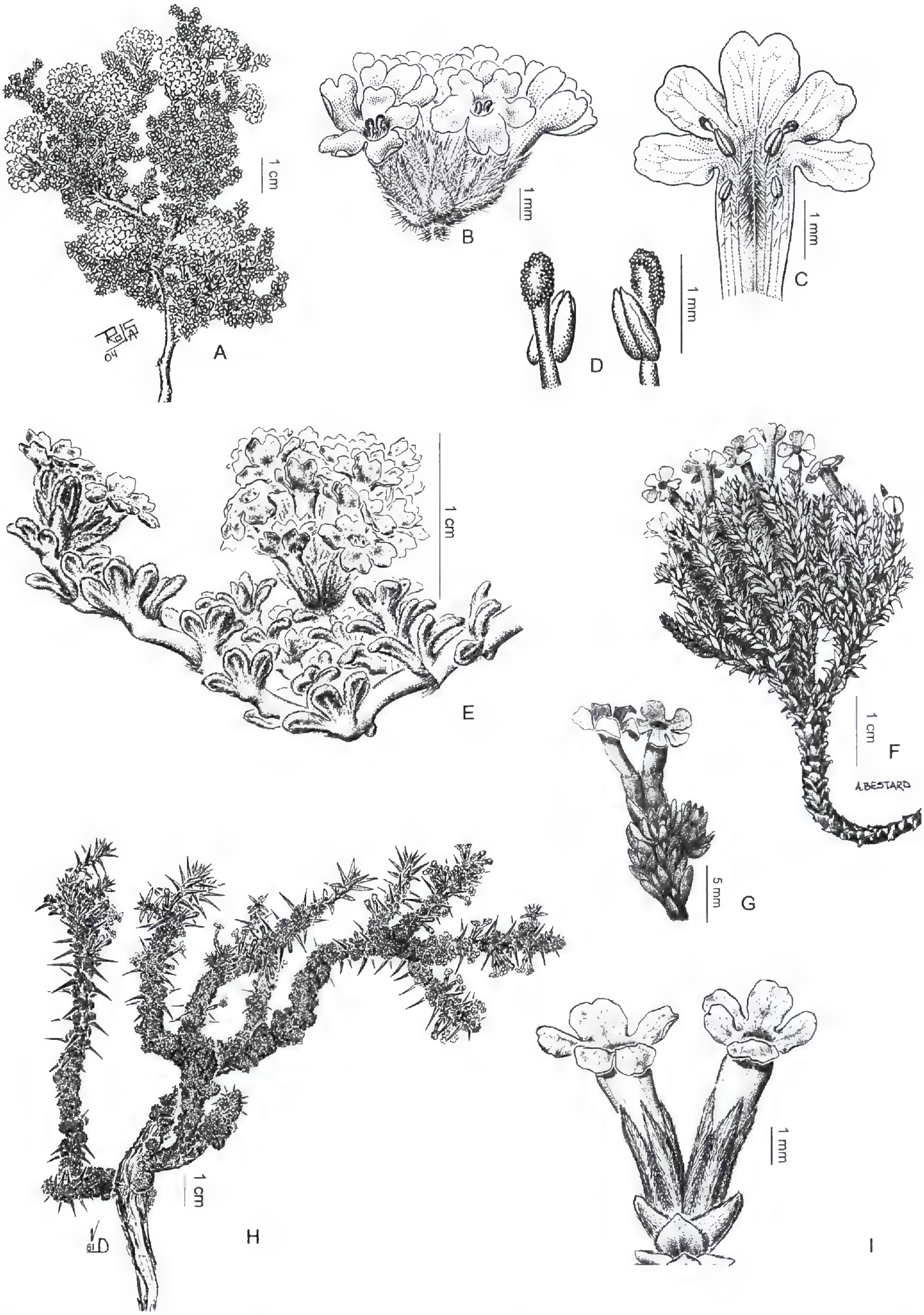


Figura 3. A–E. *Junellia micrantha*. —A. Aspecto general, con hojas enteras. —B. Inflorescencia. —C. Corola abierta. —D. Antera. A–D de *Botta 426*. —E. Aspecto general, con hojas trilobadas (de *Cabrera 3397*). F, G. *Junellia uniflora*. —F. Aspecto general. —G. Inflorescencia. F, G de *Cabrera 31136*. H, I. *Junellia seriphioides*. —H. Aspecto general. —I. Inflorescencia. H, I de *Volponi 24*.

(Speg.) Speg., Anales Soc. Ci. Argent. 53: 245. 1902. *Junellia serpyllifolia* (Speg.) Moldenke, Lilloa 5: 401. 1940. TIPO: Argentina. San Jorge, feb. 1896, *C. Ameghino LP 10405* (holotipo, LP!; isotipo, SI!).

Verbena wilczekii Briq., Annuaire Conserv. Jard. Bot. Genève 4(2): 18. 1900. *Junellia wilczekii* (Briq.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. TIPO: Argentina. Mendoza, San Rafael: Valle del Atuel, 2300–2700 m, *E. Wilczek 37* (holotipo, LAU no visto; isotipos, K!, G foto FM 24701 SI!).

Verbena chubutensis Speg., Anales Soc. Ci. Argent. 53: 245. 1902. *Junellia chubutensis* (Speg.) Moldenke, Lilloa 5: 394. 1940. TIPO: Argentina. Chubut: Corcovado, aest. 1900, *N. Illin s.n.* (holotipo, LP!; isotipo, SI!).

Caméfitos hasta 10 cm alt.; ejes hispídulos, pubescencia patente, entrenudos de 0.3–3 mm. Hojas homomorfas, de 1–4 × 1.5–2 mm, no imbricadas, trilobadas, con lóbulos espatulados o enteras, elípticas anchas hasta orbiculadas, ápice redondeado u obtuso, base cuneada, densamente estrigosas, en ambas superficies o menos densa la superficie adaxial, pelos adpresos. Monobotrios contraídos, plurifloros, de 20 a 30 flores sésiles, blancas, celestes, liláceas o rojizas, en espigas cortas, hasta 2 cm; brácteas de 1.5–2.5 × 1.5–2 mm, ovadas o elípticas, cóncavas, cara adaxial glabra, cara abaxial estrigosa. Cáliz de 2.5–3.5 mm, cilíndrico, dientes cortos, triangulares, generalmente todos iguales, superficie externa estrigosa; corola de 5–6.5 mm, glabra en la superficie externa, fauce glabra; par superior de estambres generalmente con apéndices conectivales exertos, conectivo no superando la longitud de las tecas; gineceo de 4–4.5 mm, base del estilo ensanchada. Clusas de 2–2.5 × ca. 1 mm, glabras, ápice obtuso cubierto por la base del estilo, cara dorsal reticulada, castaña, caras comisurales papilosas, blancas.

Cariología. $n = 9$; $n = 10$ (*Junellia serpyllifolia*, Botta & Brandham, 1993: 144, 145, fig. 1B, C).

Distribución geográfica. Habita en Argentina, desde el sur de Mendoza hasta Santa Cruz, crece desde la región de la costa hasta la zona cordillerana, hasta 3500 m, en la provincia fitogeográfica Patagónica (Fig. 1E).

Fenología. Florece y fructifica entre octubre y febrero.

Iconografía. Botta, 1999: 177, fig. 128, sub *Junellia serpyllifolia*.

Observaciones. Esta especie se caracteriza por la presencia de apéndices glandulares conectivales y por la corola apenas sobrepasando el cáliz. En algunos ejemplares de la región oeste de Neuquén, no se ha observado la presencia de glándulas conectivales; sin embargo los restantes caracteres de la morfología foliar y floral coinciden con los de la especie (*Schajovskoy s.n.*; SI 25625; *Roig 13058*; *Iter patagonicum 715*; *Correa 10504*).

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Biedma, 50 km S de Pto. Madryn, ruta 1, *Correa 10171* (SI). **Mendoza**: Dpto. Malargüe, Portezuelo del Ancho, 30 km W de Los Molles, *Fabris 8514* (SI). **Neuquén**: Dpto. Catan Lil, ruta 40, entre La Negra y Catan Lil, *Gentili 817* (SI). **Río Negro**: Dpto. Ñorquinco, El Portezuelo, a 25 km de El Maitén, *Ruíz Leal 26869* (SI). **Santa Cruz**: Dpto. Deseado, ruta 502, 10 km al N de los Manantiales, *Boelcke 16229* (SI).

Junellia Moldenke subgén. **Junellia** secc. **Guedesia** Botta, Darwiniana 29: 388. 1989. TIPO: *Verbena uniflora* Phil. [= *Junellia uniflora* (Phil.) Moldenke].

Verbena ser. *Pauciflorae* Briq., syn. nov. Annales Conserv. Jard. Bot. Gèneve 4(2): 17. 1899. *Verbena* secc. *Junellia* ser. *Pauciflorae* (Briq.) Tronc., Darwiniana 18: 313. 1974. *Junellia* Moldenke subgén. *Junellia* secc. *Guedesia* ser. *Pauciflorae* (Briq.) Botta, Darwiniana 29. 389. 1989. TIPO: *Verbena uniflora* Phil. [= *Junellia uniflora* (Phil.) Moldenke].

Verbena secc. *Junellia* ser. *Minutifoliae* Tronc., Darwiniana 18: 314. 1974. *Junellia* Moldenke subgén. *Junellia* secc. *Guedesia* ser. *Minutifoliae* (Tronc.) Botta, p.p., Darwiniana 29: 389. 1989. TIPO: *Verbena minutifolia* Phil. [= *Junellia minutifoliae* (Phil.) Moldenke].

Verbena secc. *Junellia* ser. *Thymifoliae* Tronc., p.p., Darwiniana 18: 314. 1974. TIPO: *Verbena thymifolia* Lag. [= *Junellia thymifolia* (Lag.) Moldenke].

Arbustos bajos o cojines. Hojas homomorfas, enteras o divididas, imbricadas o no, inermes o con ápice punzante pero no transformadas en espinas, a veces subcarnosas. Monobotrios plurifloros o pauciflloros. Apéndices conectivales no desarrollados. Número cromosómico $x = 9$.

Observación. Aquí aceptamos la sección *Guedesia*, sin las series propuestas por Botta (1989), porque los caracteres enunciados por la autora no resultan suficientes para avalar la subdivisión. Por otra parte Botta (1989: 389) consideró a *J. odonellii*, en esta sección; en la presente contribución, se ubica a la misma en la sección *Junelliopsis*.

2. Junellia aretioides (R. E. Fr.) Moldenke, Lilloa 5: 393. 1940. Basónimo: *Verbena aretioides* R. E. Fr., Nova Acta Regie Soc. Sci. Upsal, ser. 4, 1: 109. 1905, non *Verbena aretioides* Hayek, 1908. TIPO: Bolivia. "Salitre pr. Yavi (In Argentina) in montis siccis apricis", 4000 m, 6 ene. 1902, *R. E. Fries 1033* (holotipo, S no visto, S foto SI!; isotipos, GH no visto, NY!, SI!).

Verbena pygmaea R. E. Fr., Nova Acta Regie Soc. Sci. Upsal, ser. 4, 1: 108. 1905. *Junellia pygmaea* (R. E. Fr.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. TIPO: Argentina. Jujuy, Santa Catalina: La Perdida, 4100 m, in petrosis, 27 ene. 1901, *F. Kurtz 11513* (lectotipo, aquí designado, S!; isotipos, NY!, SI!).

Caméfito en placas de 1.5–2 cm de alto, con raíces gruesas; muy ramificado, ramas de 3–5 cm. Hojas homomorfas, de 2–3 × ca. 1 mm, densamente

imbricadas, tetrásticas, subcarnosas, sésiles, en general 3-lobadas, lóbulos de 1 mm, oblongos, ápice obtuso, a veces enteras, incano-pubescentes en ambas caras, cilioladas en el margen. Monobotrios paucifloros, de 2 a 3 flores; brácteas anchamente ovadas, de 2–2.5 × 1.5–2 mm, ápice obtuso o subagudo, vellosas en la cara abaxial, con pelos adpresos en la adaxial, ciliadas en el margen. Cáliz de 2.5–3 mm, velloso en la mitad superior, dientes de 0.5–1 mm, triangulares; corola blanca, de 4–5 mm, tubo algo ensanchado en el ápice, externamente glabro; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 2.5–3.5 mm, base del estilo apenas ensanchada. Clusas ca. 2 mm, glabras, subreniformes, ápice truncado y cubierto por la base del estilo, base angostada, cara dorsal de color pardo obscura, caras comisurales blancas, papilosas.

Nombre vulgar. "Yaretilla" (Botta, 1993: 76).

Distribución geográfica. Bolivia, Chile y Argentina en las provincias de Jujuy y Salta. Crece en suelos pedregosos de la provincia Altoandina, desde los 3000 y 4500 m (Fig. 1A).

Fenología. Se ha coleccionado en flor desde octubre hasta abril.

Iconografía. Botta, 1984: 348, fig. 7 (sub *Verbena pygmaea*). Botta, 1999: 75, fig. 34.

Observaciones. *Junellia aretioides* se diferencia de *J. trifurcata* (Phil.) Moldenke, porque esta última presenta los lóbulos foliares vellosos en la superficie y ápice agudo.

En la descripción original de *Verbena pygmaea* se citan dos ejemplares de Kurtz. Botta (1984: 347) cita *Kurtz 11513*, como isotipo de la especie, depositado en SI. En realidad la autora realizó una lectotipificación, sin mencionarlo, por lo que en esta oportunidad se hace efectiva la misma.

Material adicional examinado. ARGENTINA. **Jujuy**: Dpto. Tumbaya, Abra de Lipan, *Botta 356* (SI). **Salta**: Dpto. Los Andes, San Antonio de los Cobres, subida a Alto Chorrillos, *Cabrera 8270* (SI). BOLIVIA. **Potosí**: Sud López, cerro Tapaquillcah, *Liberman 202* (SI). **Tarija**: Punta Patanca, *Fiebrig 2616* (BM, K, LIL, SI). CHILE. **I Región**: Tarapacá, Tranque de Caritaya, *Ricardi 25595* (SI).

3. Junellia azorelloides (Speg.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. Basónimo: *Verbena azorelloides* Speg., Revista Fac. Agron. Univ. Nac. La Plata 3: 562. 1897. TIPO: Argentina. Santa Cruz, Dpto. Deseado: "In altiplanitie secus Golfo San Jorge", feb. 1896, *C. Ameghino s.n.* (holotipo, LP 1881!, LP foto SI!; isotipo, SI!).

Caméfito pulvinado, de 2–3 cm de alto, inerme, formando cojines subhemisféricos o chatos, muy ramificados, compactos; ramas de 2–3 cm, con entrenudos muy abreviados. Hojas homomorfas, de 2–3 × ca. 0.5 mm, lineares, enteras, sésiles, crasas, no espinosas, ápice obtuso y soldadas en la base, pubérulas en ambas superficies, con pelos más largos en la parte basal del margen. Monobotrios en espigas contraídas, plurifloras de 4 a 5 flores; brácteas de 2.8–3.5 × 1.5–2.5 mm, ápice agudo, con el nervio medio prominente en la cara abaxial y ésta villosa, cara abaxial subglabra. Cáliz tubuloso, de 6.5–7 mm, hispídulo, ápice 5-dentado, dientes breves; corola de 11.5–12 mm, rojiza o blanco-rosada, exteriormente hispídula; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo 8 mm, base del estilo ensanchada. Clusas 4 mm, glabras, ápice cubierto por la base del estilo.

Distribución geográfica. Crece en Argentina en las provincias de Mendoza, Chubut y Santa Cruz, correspondiente fitogeográficamente en el Dominio Alto-andino, provincia Patagónica, desde el nivel del mar hasta los 2000 m (Fig. 1A).

Fenología. Se ha coleccionado en flor desde noviembre a enero.

Iconografía. Botta, 1999: 167, fig. 124, 169.

Observaciones. Entre las especies de la serie, esta se caracteriza por sus pequeñas hojas lineares, pubérulas.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Ameghino, 21 km S de Garayalde, *Vallerini 2082* (SI). **Mendoza**: Dpto. San Rafael, El Sosneado, el Ángulo, *Lagiglia 8370* (SI). **Santa Cruz**: Dpto. Deseado, Deseado, *Ameghino 78* (BA).

4. Junellia congesta (Tronc.) Moldenke, Phytologia 19: 435. 1970. Basónimo: *Verbena congesta* Tronc., Darwiniana 14: 631. 1968. TIPO: Argentina. Neuquén, Dpto. Zapala: In mountains in the Zapala district, 1925–1927, *E. Opazo s.n.*, Herb. *H. F. Comber 1290* (holotipo, K!, K foto SI!).

Caméfito pulvinado denso, de 3 cm alt., ramas breves, densamente foliosas. Hojas homomorfas, de 3.5–4 mm, imbricadas, profundamente tripartidas, connadas en la base, carnosas, glabras, lóbulos linear espatulados, obtusos, enteros a veces los laterales algo divididos. Monobotrios en espigas contraídas, plurifloras de 10 a 12 flores, densos; brácteas de 5–6 mm, oblongas, subcarnosas, ciliadas en el borde. Cáliz de 5.5–6 mm, piloso en la mitad inferior y sobre los nervios, con pelos glandulares breves, dientes suba-

gudos; corola de 6.5–8 mm, pubescente en la mitad superior externa, internamente con densos pelos retrorsos, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo con estilo ensanchado. Fruto no visto.

Distribución geográfica. Se conoce hasta el presente sólo el material tipo de la especie (Fig. 1B).

Iconografía. Troncoso, 1968: 631, 632, fig. 1 sub *V. congesta*; Botta, 1999: 170, 171, fig. 125.

Observaciones. Especie afín a *Junellia digitata* de la que se diferencia por la pubescencia y la división de las hojas, las forma y división de las brácteas y tamaño de la corola.

5. Junellia digitata (Phil.) Moldenke.

5a. Junellia digitata (Phil.) Moldenke var. **digitata**, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. Basónimo: *Verbena digitata* Phil., Anales Mus. Nac. Santiago de Chile, Bot. 8: 59. 1891. TIPO: Argentina. Catamarca, Antofagasta de la Sierra: Colorados, ene. 1885, *F. Philippi s.n.* (lectotipo, aquí designado, SGO 42522!). Figura 6F.

Verbena triternata Phil., Anales Univ. Chile 90: 615. 1895. TIPO: Chile: "In Araucania habitat", La Cueva, ene. 1887, *s. col.* (holotipo, SGO 54783!, SGO foto SI!; isotipo, SI!).

Junellia spegazzinii Moldenke, Phytologia 7: 258. 1960. Nombre reemplazado: *Verbena nubigena* Speg., Comun. Mus. Nac. B. Aires. 1(4): 137. 1899, nom. illeg., non *Verbena nubigena* Poepp., Froriep's Not. Natur-Heilk. Ser. 1, 23: 292. 1829. *Verbena spegazzinii* (Moldenke) Tronc., Darwiniana 18: 314. 1974. TIPO: Argentina. Salta, Cachi: Cerro de Cachi, ene. 1897, *C. Spegazzini s.n.* (holotipo, LP 10399!; isotipo, SI!).

Junellia punctulata Hieron. & Moldenke ex Moldenke, Phytologia 3: 112. 1949. TIPO: Argentina. La Rioja, Famatina: Sierra de Famatina, Cuesta del Tocino, 2 feb. 1979, *G. H. E. W. Hieronymus & G. Niederlein 686* (holotipo, MVM no visto; isotipos, CORD!, SI!, G foto FM 24697 SI!).

Verbena tridactyla Phil., Anales Mus. Nac. Santiago de Chile, Bot. 8: 59. 1891. *Junellia tridactyla* (Phil.) Moldenke, Phytologia 2: 137. 1946. TIPO: Chile. Antofagasta: Copacoya, 19 feb. 1885, *F. Philippi s.n.* (lectotipo, designado por Acevedo de Vargas [1951: 68], SGO 54801!; isotipo, SI!).

Caméfito en placa, estolonífero, formando pequeños manchones aislados, de 3–5 cm alt.; tallos postrados con corteza ferrugínea que se desprende fácilmente y entrenudos 1 mm. Hojas homomorfas, de 3–5(–7) × 5–7 mm, subcrasas, profundamente 3-partidas (a veces 2-partidas) a veces con la parte basal angostada, plana y limbo con lóbulos oblongos de ápice redondeado, generalmente dividido, con ambas caras híspido-glandulosas, las de los macroblastos opuestas las de los braquiblastos imbricadas; pecíolo de 2 mm o menor. Monobotrios en espigas contraídas, pluriforas de 5 a 8 flores, densas; brácteas de 3–5.5 mm, 3-partidas hasta 3-sectas. Cáliz de 3–5 mm, velloso, con pelos glandulares, en la región apical; dientes breves, triangulares, subiguales; corola de 7.5–9 mm, rojiza, glabra externamente y pilosa en el interior; fauce glabra o con escasos pelos; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 4–6 mm, base del estilo ensanchada. Clusas de 2.5–3.5(–4.5) mm, glabras, ápice obtuso y cubierto por la base del estilo, base angostada, cara dorsal reticulada, de color castaño oscuro y las comisurales blancas, papilosas.

Distribución geográfica. Norte de Chile y noroeste de Argentina, desde Salta hasta San Juan, en la provincia fitogeográfica Altoandina, crece en general en suelos pedregosos, entre los 3500 y 4700 m (Fig. 1C).

Fenología. Florece y fructifica en verano.

Iconografía. Botta, 1984: 350, fig. 8, a–h.

Observaciones. Junellia digitata es afín a *J. congesta*; esta última se caracteriza por sus hojas glabras, con lóbulos enteros. Se diferencia de *J. tridactylites*, porque presenta la corola con pubescencia adpresa retrorsa, en la superficie exterior.

En la descripción original de *Verbena digitata*, Philippi (1891) cita un ejemplar de Guanaqueros. Consultado el herbario de SGO, y otros herbarios, no se localizó ningún ejemplar de esa localidad, correspondiente a esta especie. Por lo tanto se procedió a elegir un lectotipo entre los materiales estudiados por el autor, colectados por F. Philippi en su viaje a Tarapacá y depositados en SGO, lugar de trabajo del autor, eligiendo el material citado por ser el más completo.

En el caso de *Verbena tridactyla*, Acevedo de Vargas (1951: 68), la autora señaló SGO 54801, como "typus", criterio que se comparte.

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Antofagasta de la Sierra, Las Punillas, cerca de Falda de Ciénaga, 4300 m s.m., 19 feb. 1980, *Cabrera 31856* (SI). **Jujuy**: Dpto. Susques, Abra Chorrillos, 16 feb. 1980, *Cabrera 31769* (SI). **La Rioja**: Dpto. Vinchina, El Peñón, Precordillera, 3800 m s.m., 3 feb. 1947, *Hunziker J. H. 2146* (SI). **Salta**: Dpto. Los Andes, Pastos Grandes, camino a Mina La Julia, Corrida de Cori, 4500 m s.m., 17 feb. 1945, *Cabrera 8788* (LP). **San Juan**: Dpto. Iglesias, Reserva de San Guillermo, de Las Carachas al Cajoncito (Cajón Chico), ca. 3500 m s.m., 22 feb. 1984, *Kiesling 4629* (SI). **Tucumán**: Dpto. Tafí, Cumbres Calchaquíes, cerro Negrito, 4200 m s.m., 3 ene. 1953, *Petersen 867* (SI). CHILE. **I Región**: Iquique, Mamiña, 15 abr. 1985, *s. col.*, SGO

68385 (SGO). **II Región**: Antofagasta, inter Vegas del Diablo & Antofagasta, ene. 1885, *Philippi s.n.* (SI); Desertum Atacama, feb. 1888, *Philippi s.n.* (K).

5b. Junellia digitata var. **integerrima** (Botta) Botta, Hickenia 2: 128. 1995. Basónimo: *Verbena digitata* var. *integerrima* Botta, Darwiniana 25(1–4): 350. 1984. TIPO: Argentina. Jujuy, Susques: lomás al pie del Cerro Tuzgle, 2 feb. 1944, *A. L. Cabrera 8401* (holotipo, LP!; isotipo, SI!). Figura 6G.

Difiere de la variedad típica por tener brácteas enteras, de igual o menor longitud que el cáliz.

Distribución geográfica. En Argentina, en la provincia de Jujuy y en Chile en las regiones I y II. Habita suelos pedregosos aproximadamente entre los 3200 y 4450 m, en el límite de las provincias fitogeográficas Altoandina y Prepuneña (Fig. 1C).

Fenología. Florece y fructifica en verano.

Material adicional examinado. ARGENTINA. **Jujuy**: Dpto. Susques, Planicie al S del Cerro Tuzgle, *Werner 123* (SI). CHILE. **I Región**: Iquique, Collaguasi, Quebrada La Represa, *Teiller 3082* (CONC). **II Región**: El Loa Ojo de San Pedro, *Pisano 1792* (CONC).

6. Junellia minima (Meyen) Moldenke, Prelim. Alph. List Inval. Incor. Sci. Names Verben. Avicen.: 47. 1940. Basónimo: *Verbena minima* Meyen, Reise Erde 1: 451. 1834. TIPO: "Pérou, sur le gran plateau", Weddell, H. A. 1857–1861. Chlor. And. 2: 154–155, pl. 62B (neotipo, aquí designado).

Junellia straguloides Moldenke, Phytologia 1: 483. 1941. TIPO: Argentina. Jujuy: Tilcara, valleys above San Gregorio, 14,000 ft., 11 Feb. 1939, *E. K. Balls 6000* (holotipo, US no visto, US foto SI!; isotipo, K!, K foto SI!).

Junellia minima var. *strigosa* Moldenke, Phytologia 5: 340. 1956. TIPO: Argentina. Mendoza: Las Heras, Cruz de Paramillo, 3 ene. 1950, *Melis y Paci 279* (holotipo, NY no visto; isotipo, SI!).

Caméfitos en placas, de 1–5 cm de alt., con raíces leñosas. Hojas homomorfas, de 4–5 × 1–1.5 mm, lineares o angostamente ovadas, por lo general enteras, a veces las inferiores 2–3-fidas, sésiles, densamente imbricadas, connadas en la base, subcóncavas, subcarenadas, rígidas, generalmente mucronadas, a veces espinescente en el ápice y algo incurvo, cara abaxial con el nervio medio prominente, cara adaxial densamente pubescente hacia el ápice, margen ciliado. Monobotrios de 2 a 3 flores; brácteas de 2.5–3.5 × 1.5–2.5 mm, ovadas hasta anchamente obovadas, cóncavas, de ápice agudo casi obtuso, pubescente en su cara abaxial, margen ciliado. Cáliz de 2.5–3 mm, pubérulo en su mitad superior, dientes subiguales, triangulares, subobtusos, ca. 0.5 mm; corola de 3.5–4.5(–5.5) mm, blanca o lilacina, glabra en el exterior, tubo corolino recto; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 2.5–3 mm, base del estilo ensanchada. Clusas de 2–2.5 mm, glabras, ápice obtuso y cubierto por la base del estilo, base angostada, cara dorsal reticulada, pardo-obscuro, caras comisurales papilosas, blancas.

Cariología. $2n = 18$ (*Junellia minima*, Botta y Brandham, 1993: 147, fig. 2D, E).

Nombre vulgar. "Kohtta" (Lilloa 5: 398. 1940); "césped de la puna" (de *Cárdenas 3740*) "yareta", "yaretilla" (Botta, 1993: 74); "hareta" (Moldenke & Moldenke, 1949: 7).

Distribución geográfica. Perú, Chile, Bolivia y Argentina. Crece entre los 2800 y 4600 m, en suelos pedregosos de la Provincia Altoandina (Fig. 1E).

Fenología. Florece y fructifica de noviembre a febrero.

Iconografía. Weddell, 1857–1861: pl. 62 (sub *Verbena minima*); Ancibor, 1980: 164, fig. 1; 182, lám. 2; 184, fig. 5A–E (sub *V. minima*).

Observaciones. Especie muy afín a *Junellia uniflora* de la que se diferencia por la morfología de la hoja, distribución de la pubescencia en el cáliz y las superficies comisurales de las clusas. No se han registrado hasta el presente de ejemplares que corroboren la presencia de esta especie en Chile, ya que Tacna, localidad donde se colectó el ejemplar *Wederman 1120*, actualmente pertenece a Perú. Por otra parte el ejemplar de *Budin 7441* (*LIL 32890*, SI), publicado por Moldenke (1944: 371) como *J. hayekii* Moldenke, se considera aquí *J. minima*.

El holotipo de Perú, Tacora, abril 1831, *Meyen s.n.*, depositado en B, fue destruido. Debido a la ausencia de isotipos, fue necesaria la neotipificación; se designó la lámina de Weddell por tener claramente representados los caracteres que caracterizan la especie (Greuter et al., 2000: Art. 8.1).

Con respecto a *Verbena nubigena* Poepp. (Froriep's Not. Natur-Heilk., Ser. 1, 23: 292. 1829), el autor no indica ejemplar de referencia, se ha considerado que podría ser un sinónimo de *Junellia digitata*, teniendo en cuenta la descripción original.

Verbena straguloides (Moldenke) Tronc. (Darwiniana 18: 313. 1974) no era validamente publicado, porque no hay referencia a la cita bibliográfica donde se describió la especie.

Material adicional examinado. ARGENTINA. **Jujuy**: Dpto. Cochinoca, Abra de la Cruz, camino a Casa Colorada, *Dell'Arciprete s.n.*, BACF 2324 (BACF, SI). **La Rioja**: Dpto. Gral. Sarmiento, Reserva Laguna Brava, Peñas Negras,

campamento de la empresa minera El Dorado, *Biurrun 4555* (SI). **Mendoza**: Dpto. Las Heras, Paramillos de Uspallata, *Carette s.n.*, MERL 2564 (MERL, NY). **San Juan**: Dpto. Calingasta, Del Observatorio El Leoncito al Portezuelo del Tontal, *Kiesling 4674* (SI). **Tucumán**: Dpto. Tafí, Cerro Muñoz, Las Ánimas, *Castillón 3265* (LIL). BOLIVIA. **La Paz**: Murillo, La Paz, El Alto, borde de la ciudad, *Beck 3980* (SI). **Oruro**: Avaroa, De Challapata hacia Potosí, Ventilla, 14 km antes del cruce hacia Macha, *Beck 14110* (SI). **Potosí**: Frías, Tres Palcas (atocha Villazán), *Cardenas 3740* (SI). **Puna**: Julé, *Shepard 26* (NY). CHILE. **I Región**: Arica, camino de Putre a Chucuyo, Km 17, *Marticorena 203* (CONC). PERÚ. **Arequipa**: Cailloma, sobre Chivay, *Weberbauer 6895* (F). **Moquegua**: Cordillera above Torata, *Weberbauer 7472* (F). **Puno**: Juliaca, *Stafford 1136* (F). **Tacna**: Tacna, Cordillera Volcán Tacara, Ancara, *Werdermann 1120* (SI).

7. Junellia patagonica (Speg.) Moldenke, Lilloa 5: 399. 1940. Basónimo: *Verbena patagonica* Speg., Anales Soc. Ci. Argent. 15: 114. 1883. TIPO: Argentina. Santa Cruz: Río Santa Cruz, Cerro de los Caracoles, Patagonia Austral, ene. 1882, *C. Spegazzini s.n.* (holotipo, LP!; isotipo, SI!).

Verbena morenonis Kuntze, Revis. Gen. Pl. 3(2): 256. 1898. TIPO: Argentina. "Patagonia, s.f.", *F. Moreno & Tonini 176* (holotipo, NY!).

Caméfitos pulvinados hasta 5 cm alt., densísimos, a veces erectos, verde-claros. Hojas homomorfas, de 1–2 × 1–2 mm en la base, ovadas, enteras, revolutas, carnosas, con pubescencia adpresa-retrorsa y un mechón importante de pelos canos en la base interior. Monobotrios plurifloros hasta de 10 flores, flores perfumadas, brácteas de 3–3.5 mm, ovadas, ápice agudo, tomentosas. Cáliz de 5–6 mm, tomentoso, adpreso-antrorso, a veces adpreso-retrorso, 5 dientes agudos, 2 más cortos que los otros; corola de 12–12.5 mm, blanca, rosada, amarilla, rojo violáceo y colores intermedios, perfumada, pubescencia pilosa a sericea, adpreso-retrorsa exteriormente; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas, gineceo de 7.5–8 mm, base del estilo ensanchada. Clusas 3.5–4 mm, ápice cubierto por la base del estilo, cara dorsal reticulada.

Cariología. $n = 9$ (*Junellia patagonica*, Botta & Brandham, 1993: 147, fig. 2A).

Distribución geográfica. Especie abundante en la provincia fitogeográfica Patagónica. Crece alrededor de los 1900 m (Fig. 1F).

Fenología. Florece en primavera.

Iconografía. Botta, 1999: 176, 180, fig. 133.

Observaciones. Esta especie se caracteriza por la forma de las hojas y el mechón de pelos canos en la base de las mismas, además por formar cojines inermes con flores intensamente perfumadas. En algunos ejemplares por ejemplo *Donat 221* (BM, K, MO, SI), *Liberman 4300* (MO), *Vallerini 3430* (SI), *Ruíz Leal 25711* (SI), se ha observado la presencia de hojas trilobadas, siendo los restantes caracteres propios de la especie.

Verbena thymoides Phil. ex Ball (J. Linn. Soc. 27: 497. 1891) es un homónimo posterior. La descripción del mismo es muy incompleta y además no se ha podido localizar el material de Andrews; sin embargo en K, existe un ejempar de *Darwin 225*, de Porto Desiderato, que se ha determinado como *Junellia patagonica*, y podría ser el referido en la descripción original: "A plant which appears identical with this was collected at Port Desire by Darwin". Por esta razón podría tratarse de un sinónimo de *J. patagonica*.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Languiñeo, cerca de Ea. Quichana, *Cabrera 33118* (SI). **Neuquén**: Dpto. Añelo, sa. Auca Mahuida, 1000 m al W de la antena repetidora, *Rossow 1615* (BAB, SI). **Río Negro**: Dpto. Valcheta, Somoncurá, lag. Raimunda, *Correa 6250* (BAB). **Santa Cruz**: Dpto. Deseado, a 17 km de la entrada a Ea. Romberg, *Correa 10284* (BAB, SI).

8. Junellia silvestrii (Speg.) Moldenke, Lilloa 5: 401. 1940. Basónimo: *Verbena silvestrii* Speg., Anales Soc. Ci. Argent. 53: 243. 1902. TIPO: Argentina. Santa Cruz: "secus río Santa Cruz", feb. 1900, *F. Silvestri s.n.* (lectotipo, designado por Botta (1989: 389), LP!; isotipo, SI!).

Caméfitos pulvinados de 3–4 cm alt., inermes, muy duros, ramosísimos, glabros. Hojas homomorfas, de 2–3(–5) × 1 mm, enteras, ovadas, imbricadas, tetrásticas, carnosas, glabras o ciliadas en el margen y en la base. Monobotrios bifloros, brácteas de 2–2.5 mm, ovadas, inflexas en el ápice, glabras, a veces ciliadas. Cáliz de 4 mm long., tubuloso, 5-costado, 5-dentado, dientes reducidos, subtriangulares, glabro; corola de 6–8 mm, tubo recto piloso o a veces glabro, limbo patente, 5-lobado; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6 mm, la base del estilo ensanchada. Clusas de 3 mm, glabras, ápice cubierto por la base del estilo, cara dorsal apenas reticulada, caras comisurales blancas con papilas cortas.

Distribución geográfica. Argentina y Chile. Crece en la provincia fitogeográfica Patagónica, distritos del Golfo de San Jorge y Patagónico occidental y provincia Subantártica, distrito Magallánico (Fig. 2B).

Fenología. Florece en primavera y verano.

Iconografía. Botta, 1999: 183, fig. 135.

Observaciones. Entre las especies de la serie, se caracteriza por sus hojas glabras, brillantes y dispuestas en forma imbricada.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Florentino Ameghino, Begoña, *Soriano 2065* (SI). **Santa Cruz**: Dpto. Deseado, camino Jaramillo–bosque petrificado, *Crespo 1759* (BAB, SI). CHILE. **XII Región**: Magallanes, Sierra Cazador, *Magens 19090* (SI).

9. Junellia succulentifolia (Kuntze) Moldenke, Lilloa 5(2): 402. 1940. Basónimo: *Verbena succulentifolia* Kuntze, Revis. Gen. Pl. 3(2): 257. 1898. TIPO: Argentina. Patagonia, s.f., *F. Moreno y Tonini* 335 (holotipo, NY no visto; isotipo, LP!, B† foto FM 17449 SI!).

Verbena scabridoglandulosa Turrill, Curtis Bot. Mag. 167(1): tab. 98. 1950. *Junellia scabridoglandulosa* (Turrill) Moldenke, Phytologia 3: 310. 1950. TIPO: Argentina. Neuquén, Huiliches: Malleo, 7 ene. 1927, *H. F. Comber 957* (holotipo, K!, K foto SI!).

Arbustos bajos de 15 cm alt., inermes, globosos, escabrosos con pubescencia glandular corta y pelos simples en tallos, hojas y flores, entrenudos de 5–30 mm. Hojas homomorfas, de 4 × 1.5 mm, sésiles, opuestas, linear-oblongas, obtusas, brevemente lobadas en la base, a veces hojas trilobadas y enteras, carnosas, margen revoluto. Monobotrios en espigas contraídas densas, plurifloras de 10 a 15 flores, alargadas en la madurez, de 5–30 × 4–15 mm; brácteas de 6 mm, elípticas, ápice agudo, pubescentes. Flores blanco-azuladas a violáceas, intensamente perfumadas. Cáliz 9 mm, con pelos cortos, rígidos; corola de 12 mm, tubo recto, exteriormente con pelos cortos retrorsos, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6.5–7 mm, base del estilo ensanchada. Clusas de 4 × 1–2 mm, glabras, ápice cubierto por la base del estilo, cara dorsal castaña, estriada, caras comisurales blancas, papilosas, margen lateral engrosado, formando una pequeña ala.

Cariología. $2n = 18$ (*Junellia succulentifolia*, Botta & Brandham, 1993: 147, fig. 2B).

Nombre vulgar. "Yerba de la perdiz" (de *Offerman s.n.*, SI).

Distribución geográfica. Habita en la provincia fitogeográfica Patagónica, desde La Pampa a Chubut; crece desde el nivel del mar hasta los 1110 m (Fig. 2C).

Fenología. Florece en primavera y verano.

Iconografía. Botta, 1999: 182, 187, fig. 139.

Observaciones. Se caracteriza por ser un arbusto bajo, con hojas carnosas, con pelos glandulares, lo que da a la planta un aspecto viscoso. Es afín a *Junellia thymifolia*, de la que se diferencia por la pubescencia de las hojas.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Florentino Ameghino, 1 km antes del dique F. Ameghino, desde Dolavon, *Correa 10196* (SI). **La Pampa**: Dpto. Curáco, entre Lihuel Calel y Puelches, *Cano 532* (SI). **Neuquén**: Dpto. Catán Lil, Sierra de Catán Lil, *Cabrera 32971* (SI). **Río Negro**: Dpto. Avellaneda, 70 km al NW de los Menucos, *Ruíz Leal 26345* (SI).

10. Junellia thymifolia (Lag.) Moldenke, Phytologia 2(2): 51. 1941. Basónimo: *Verbena thymifolia* Lag., Gen. Sp. Pl. 18. 1816. TIPO: Argentina. Santa Cruz, Lago Buenos Aires: ruta 40 y Valle del río Deseado, 1 feb. 1949, *A. Soriano 3406* (neotipo, aquí designado, SI!).

Arbustos bajos, erectos, tallos con pubescencia adpreso-retrorsa, en general con desarrollo de braquiblastos, poco o nada ramificados. Hojas homomorfas, de 2–4.5 mm, enteras, oblongas, carnosas, márgenes recurvos y con un falso ojal en el dorso, híspidas y con algunos pelos largos blanquecinos. Monobotrios, en espigas contraídas, de 10 o 11 flores en el ápice de macroblastos; brácteas de 4–4.5 mm. Cáliz de 7–8 mm, dientes de 1–1.2 mm, pequeños, subiguales, pubescencia densa, adpresa-retrorsa; corola de 15 mm, lila o azul, pubescencia híspida, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 7.5–8 mm, base del estilo ensanchada. Clusas de 4 mm, glabras, cilíndricas, ápice obtuso y cubierto por la base del estilo, cara dorsal reticulada especialmente en el ápice, caras comisurales papilosas.

Distribución geográfica. Especie abundante en Neuquén, Chubut y Santa Cruz, y en Chile en la XII Región, en la provincia fitogeográfica Patagónica donde se encuentra en zonas áridas y en médanos (Fig. 2E).

Fenología. Florece y fructifica de octubre a enero.

Iconografía. Botta, 1999: 184, 187, fig. 138.

Observaciones. Esta especie se caracteriza por la presencia de pelos blanquecinos largos y la ausencia de pelos glandulares, carácter que la diferencia de *Junellia succulentifolia*.

Como neotipo se eligió un material proveniente del mismo lugar que la colección original, citado por Botta (1989: 390).

Sprengel (1825: 748) cita la especie para Paraguay, dato que se repite en el Index Kewensis (Hooker & Jackson, 1977: 1179). La expedición de Malaspina, nunca pasó por Paraguay: desde Europa llegaron a Uruguay y desde allí continuaron hacia el sur, llegando a Puerto Deseado, por lo que la cita para Paraguay es incorrecta. En el caso de *Verbena thymifolia* el nombre fue usado por Philippi (1895:

613) con duda (?), y amplía la descripción, que coincide con los materiales observados citando la especie para Lago Santa Cruz (actualmente Argentina).

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Biedma, Península de Valdéz, *Frazier s.n.*, BA 70387 (BA); Dpto. Escalante, Pampa del Castillo, *Cabrera 33251* (SI); Dpto. Paso de Indios, a 51 km SW ruta 24, Ea. La Marfil, *Arroyo 212* (SI); Dpto. Río Senguerr, 130 km al E del Valle Huemules, *Soriano 3211* (SI); Dpto. Sarmiento, entre Hermitte y Valle Hermoso, *Cabrera 33261 1/2* (SI); Dpto. Tehuelches, Apeleg, *Soriano 5043* (S). **Neuquén**: Dpto. Huiliches, Junín de los Andes, Cerro Santa Julia, *Schajovscoy 79/VII* (SI). **Santa Cruz**: Dpto. Deseado, ruta 281, 29 km al W de Puerto Deseado, *Botta 472* (SI); Dpto. Lago Buenos Aires, ruta 40, 33 km al N del empalme con ruta 43, *Botta 534* (SI); Dpto. Río Chico, ruta 28, 5 km de Gob. Gregores a las Horquetas, valle del río Chico, 48°24′S, 70°40′W, *Boelcke 12787* (SI); Dpto. Magallanes, San Julián, *Blake 424 A* (SI). CHILE. **XII Región**: Magallanes, *Gunckel 70300* (CONC).

11. Junellia tridactylites (Lag.) Moldenke, Lilloa 5(2): 402. 1940. Basónimo: *Verbena tridactylites* Lag., Gen. Sp. Pl. 19. 1816. TIPO: Argentina. Santa Cruz: Lago Argentino, ruta Nac. 40, 7 km al N del empalme con el camino a Calafate, 1 ene. 1993, *S. M. Botta 517* (neotipo, aquí designado, SI!).

Verbena struthionum Speg., Anales Soc. Ci. Argent. 15: 116. 1883. *Junellia struthionum* (Speg.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 177. 1942. TIPO: Argentina. Santa Cruz: Río Santa Cruz, 1882, *C. Spegazzini 1122* (holotipo, LP!, LP foto SI!; isotipo, SI!).

Verbena minutifolia Phil., Anales Univ. Chile 90: 694. 1895. Nombre remplazado: *Verbena microphylla* Phil., Linnaea 29: 21. 1857, nom. illeg., non *Verbena microphylla* Kunth, Nov. Gen. Sp. 2: 272. 1818. *Junellia minutifolia* (Phil.) Moldenke, Lilloa 5(2): 399. 1940. TIPO: Chile. Cordillera, ene. 1856, *Germain s.n.*, SGO 54784 (holotipo, SGO!, SGO foto SI!; isotipos, F no visto, SI!).

Verbena prichardii Rendle, J. Bot. 43. 33. 1905. Nombre reemplazado: *Verbena bonariensis* Rendle, J. Bot. 42: 370. 1904, nom. illeg., non *Verbena bonariensis* L., Sp. Plant. 1: 20. 1753. TIPO: Argentina: South Patagonia, lake Argentino, slopes of Mt. Buenos Aires, 1901, *Prichard s.n.* (holotipo, BM!).

Junellia rosulata Moldenke f. *rosulata*, Phytologia 2: 135. 1946. TIPO: Argentina. Chubut: Tecka, 25 dic. 1945, *T. Meyer 9586* (holotipo, NY no visto; isotipos, LIL!, SI!).

Junellia rosulata Moldenke f. *alba* Moldenke, Phytologia 2: 136. 1946. TIPO: Argentina. Chubut: Ea. "La Mimosa", 25 dic. 1946, *T. Meyer 9587* (holotipo, NY no visto; isotipo, LIL!).

Caméfitos pulvinados, postrados o erectos de 15–20 cm alt., en cojín o carpeta, laxos, inermes; ramas foliares con entrenudos de 5–6 mm, pubescencia híspida. Hojas homomorfas, de 3–5 mm, trilobadas a tripartidas, carnosas, pubescentes. Monobotrios en espigas contraídas, plurifloras de 10 a 15 flores; brácteas de 4 × 2 mm, ovadas, pubescentes, de bordes recurvos. Cáliz de 7–7.5 mm, dientes de 1.5 mm, desiguales, pubescencia generalmente densa, adpreso-retrorsa; corolas de 10–12 mm, perfumadas, blancas, violáceas, rojizas y/o amarillas, pubescencia densa adpreso-retrorsa, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 9–11 mm, base del estilo ensanchada. Clusas de 4.6 × 1.1 mm, glabras, ápice obtuso y cubierto por la base del estilo, cara dorsal reticulada.

Cariología. $2n = 18$ (sub *Junellia* aff. *minutifolia*, Botta & Brandham, 1993: 144, fig. 2C).

Distribución geográfica. Argentina, desde el sur de Mendoza hasta Santa Cruz, y Chile. En la provincia fitogeográfica Patagónica y áreas limítrofes del Monte, crece entre los 2300 y 2350 m (Fig. 2E).

Fenología. Florece en diciembre.

Iconografía. Botta, 1999: 174, 177, fig. 129.

Observaciones. El color de las flores es muy variable: se han observado en la espiga flores centrales blancas y marginales violáceas. Es probable que la coloración las flores varie en la relación con la madurez de las mismas. Esta especie se caracteriza por la pubescencia del cáliz, abundante y adpreso-retrorsa, al igual que la parte exterior de la corola. Es afín a *Junellia thymifolia*, de la que se diferencia principalmente por la pubescencia en las flores.

Se designa como neotipo el ejemplar de Botta por ser el más completo y tener como lugar de colección uno de los puntos donde arribó la Expedición Malaspina (ver comentario sub *Junellia thymifolia*).

Lagasca en su descripción original, cita como hábitat de *Verbena tridactylites* las Islas Malvinas, lo cual, a pesar que Née coleccionó allí, la especie no proviene de ese lugar ya que el tipo de vegetación de las islas es totalmente diferente. Además, la distribución más austral de la Tribu Verbenae es la provincia de Santa Cruz.

Por *Verbena philippiana* Kuntze (Revis. Gen. Pl. 3(2): 257. 1898), el autor designa un nuevo nombre para *V. microphylla* Phil., cuando ya existía un nombre reemplazante para esta especie.

Por *Verbena patagonica* Moldenke (Phytologia 1: 171. 1935), non *V. patagonica* Speg., Moldenke usa este nombre, siendo éste un homónimo posterior y además lo designa como nombre reemplazante para *V. bonariensis* Rendle, que ya tenía un nombre asignado.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Futaleufú, entrando hacia ruta a Jaramillo, *Correa 10399* (SI). **Mendoza**: Dpto. Malargüe, Portezuelo del Choique, *Ruíz Leal 21434* (SI). **Neuquén**: Dpto. Chos Malal, Extremo NW de la Pampa Ferraina, *Boelcke 11377* (BAA,

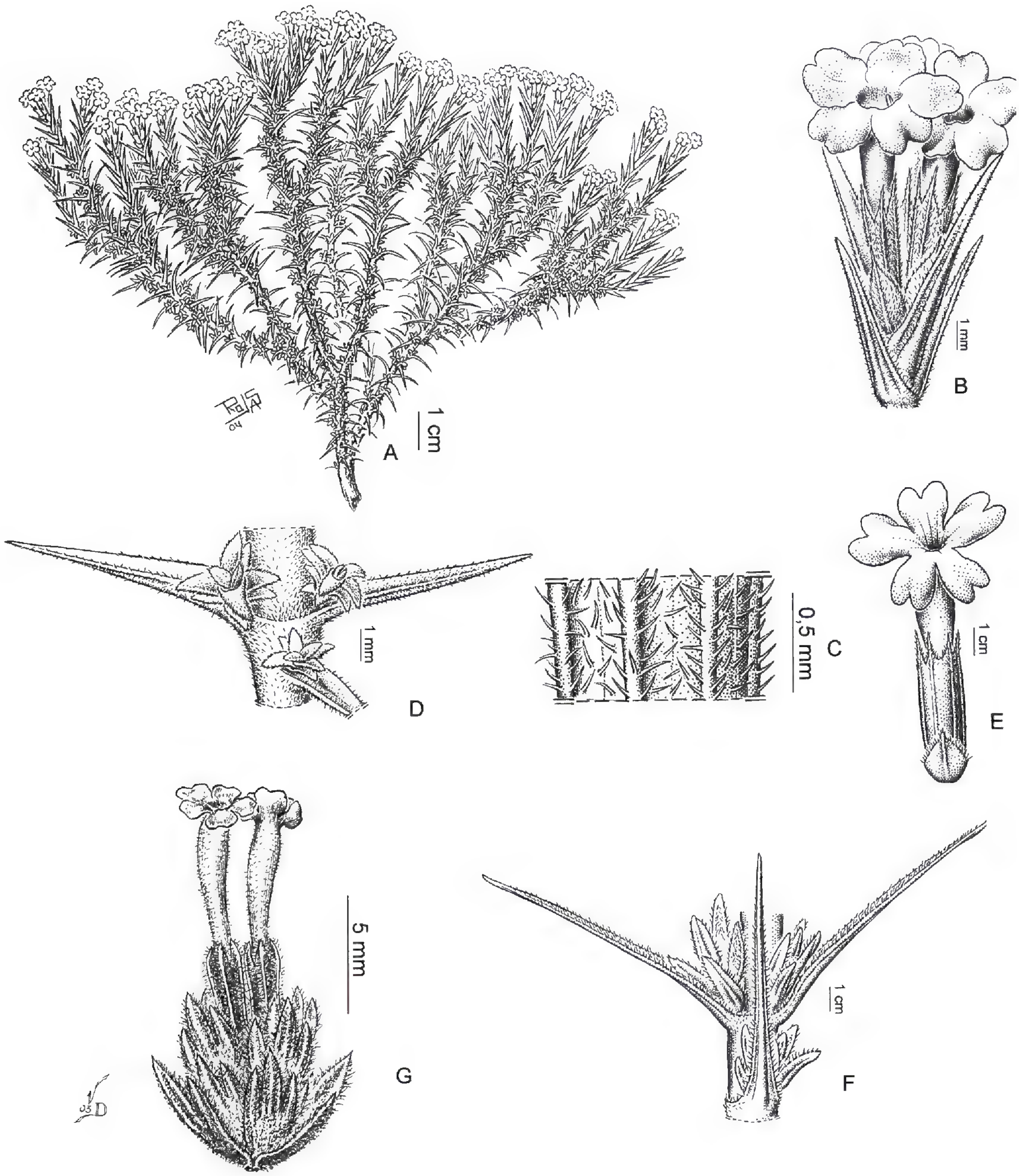


Figura 4. A–D. *Junellia toninii* var. *toninii*. —A. Aspecto general. —B. Detalle de inflorescencia. —C. Detalle de la pubescencia en el cáliz. —D. Detalle de un nudo. A–D de *Cabrera 33013*. E, F. *Junellia toninii* var. *mulinoides*. —E. Flor. —F. Detalle de un nudo. E, F de *Ruíz Leal 26359*. —G. *Junellia trifurcata*. Detalle de inflorescencia (de SGO 54802).

BAB, SI). **Río Negro**: Dpto. Ñorquinco, cerca de Río Chico, hacia Ñorquinco, *Cabrera 33067* (SI). **Santa Cruz**: Dpto. Corpen Aike, ruta, 77 km al S de río Chico, *Botta 501* (SI). CHILE. **XII Región**: Magallanes, última Esperanza, Cerro Castillo, *Boelcke s.n.*, TBPA 477 (BAB).

12. Junellia trifurcata (Phil.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. Basónimo: *Verbena trifurcata* Phil., Linnaea 29: 21. 1857. TIPO: Chile. Cordillera de Santiago, feb. 1857, *Germain s.n.*, SGO 54802 (lectotipo, designado por Botta (1989: 389), SGO!, SGO foto SI!). Figura 4G.

Arbusto hasta de 10 cm de alt., cespitoso y muy ramificado. Hojas homomorfas, de 3–5.5 × 1.5–3 mm, imbricadas, sésiles, no carnosas, tripartidas, lóbulos lineares con margen revoluto, agudos y punzantes, villosos en ambas superficies; monobotrios en espigas bifloras; brácteas ca. 5 mm, ovadas, enteras, villosas. Cáliz de 6–8.5 mm, villoso, dientes triangulares ca. 2–3 mm; corola de 10–11.5 mm, exteriormente pubescente, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6 mm, base del estilo ensanchada. Clusas de 3 mm, glabras,

ápice cubierto por la base del estilo, cara dorsal reticulada, caras comisurales papilosas.

Distribución geográfica. Chile, en la cordillera cercana a Santiago, en la provincia fitogeográfica Chilena Central (Fig. 2F).

Observaciones. Especie afín a *Junellia aretioides*; esta última presenta hojas trilobadas, lóbulos redondeados.

Material adicional examinado. CHILE. **Región Metropolitana**: Cordillera cercana a Santiago, 1856, *Germain s.n.*, SGO 42483 (SGO, SI); Cordillera del Yeso, Dec. 1873, *Reed s.n.* (K, foto FM 17455 SI).

13. Junellia uniflora (Phil.) Moldenke, Lilloa 5: 402. 1940. Basónimo: *Verbena uniflora* Phil., Linnaea 29: 20. 1857. TIPO: Chile. Santiago, dic. 1856, *C. Gay 1080* (holotipo, SGO 54800!: isotipos, P!, SI!). Figura 3F, G.

Verbena uniflora var. *glabriuscula* Kuntze, Revis. Gen. Pl. 3(2): 258. 1898. *Junellia uniflora* var. *glabriuscula* (Kuntze) Moldenke, Lilloa 5: 403. 1940. TIPO: Argentina. Patagonia, *F. Moreno & Tonini 294* (lectotipo, aquí designado, NY!; isotipo, SI!).

Caméfitos en placas densísimas, de 3–4 cm alt. Hojas homomorfas, de 2.5–5 × 1.3 mm, carnosas, imbricadas, enteras, lineares, obtusas, inermes o con el ápice punzante pero no transformadas en espinas, hirsutas en ambas caras, ciliadas, nervio medio prominente en la cara adaxial. Monobotrios de 1 a 2 flores; brácteas ca. 3 × 2 mm, anchamente ovadas, ciliadas, ápice agudo. Cáliz de 5 mm, con 5 dientes triangulares, pubescencia hirsuta; corola de 8.5–9 mm, glabra exteriormente; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6–7 mm, base del estilo ensanchada. Clusas de 3.5 × 1 mm, glabras, cilíndricas, ápice obtuso y cubierto por la base del estilo, cara dorsal reticulada, caras comisurales lisas o con escasas papilas pequeñas en los márgenes.

Nombre vulgar. "Jazmín del campo" (de *Kiesling 3227*, SI).

Distribución geográfica. Crece en Argentina en las provincias de San Juan, Mendoza, Neuquén, en Chile en las Regiones Metropolitanas, III, IV, V y VII, en la provincia fitogeográfica Altoandina, entre los 2500 y 4000 m (Fig. 2F).

Fenología. Se ha colectado en flor en el mes de enero.

Iconografía. Sanzin, 1919: 109, fig. 13 (sub *Verbena uniflora*); Böcher et al., 1963–1972: 26, fig. 13 (sub *Verbena uniflora*); Böcher, 1979: 8, 9, fig. 1, 2 (sub *Verbena uniflora*); Botta, 1999: 199, fig. 144.

Observaciones. Especie afín a *Junellia minima*; se diferencia por la pubescencia del cáliz y la superficie de las caras comisurales en las clusas.

En la descripción original de *Junellia uniflora* var. *glabriuscula*, se citan dos ejemplares, de los cuales se eligió *F. Moreno y Tonini 294* por ser el más completo.

Material adicional examinado. ARGENTINA. **Mendoza**: Dpto. San Carlos, 4 km W, sobre camino a Laguna Diamante, *Boelcke 10006* (SI). **Neuquén**: Dpto. Chos Malal, Cajón de Arroyo del Cruce, faldeo S del macizo del Domuyo, *Boelcke 11283* (SI). **San Juan**: Dpto. Calingasta, paso desde Cuesta del Río Blanco hacia el Valle de Los Patos Norte, *Kiesling 7774* (SI). CHILE. **Región Metropolitana**: Prov. Aconcagua, Cordillera de Santiago, Mercedario, *Grandjot 36a* (SI). **III Región**: Atacama, Vallenar, *Johnston 6050* (BA). **IV Región**: Ovalle, Cordillera de Ovalle, Quebrada Larga, nacimiento, *Jiles 2957* (SI). **V Región**: Los Andes, Laguna Inca, Portillo, *Arroyo 81273* (CONC). **VII Región**: Cordillera de Linares, *Philippi s.n.*, SI 3447 (SI).

Junellia Moldenke subgén. **Junellia** secc. **Junelliopsis** (Botta) P. Peralta & Múlgura, comb. nov. *Junellia* subgén. *Thryothamnus* secc. *Junelliopsis* Botta, p.p., Darwiniana 29: 392. 1989. TIPO: *Verbena bryoides* Phil. [= *Junellia bryoides* (Phil.) Moldenke].

Verbena secc. *Verbenaca* ser. *Erinaceae* Walp., p.p., Reper. Bot. Syst. 4: 14. 1845, syn. nov. *Verbena* secc. *Junellia* ser. *Erinacea* (Walp.) Tronc., Darwiniana 18: 312. 1974. *Junellia* subgén. *Junellia* secc. *Junellia* ser. 1 *Erinaceae* (Walp.) Botta, Darwiniana 29: 386. 1989. TIPO: *Verbena erinacea* Gillies & Hook. ex Hook. [= *Junellia erinacea* (Gillies & Hook. ex Hook.) Moldenke].

Verbena secc. *Verbenaca* ser. *Seriphioideae* Walp., p.p., Repert. Bot. Syst. 4: 14. 1845, syn. nov. *Verbena* subgén. *Junellia* ser. *Seriphioides* Walp. emend. Tronc., p.p., Darwiniana 18: 312. 1974. *Junellia* subgén. *Junellia* secc. *Junellia* ser. 2 *Seriphioideae* (Walp. emend. Tronc.) Botta, Darwiniana 29: 386. 1989. TIPO: *Verbena seriphioides* Gillies & Hook. ex Hook. [= *Junellia seriphioides* (Gillies & Hook. ex Hook.) Moldenke].

Arbustos bajos o cojines. Hojas homomorfas, todas espiniformes u hojas dimorfas, las de los macroblastos opuestas, enteras o partidas, espiniformes o al menos punzantes, las de los braquiblastos tetrásticas, carnosas, enteras o partidas. Monobotrios pauci- o plurifloros. Anteras sin apéndices conectivales. Número cromosómico $n = 10$.

Observaciones. Se pudo analizar material más completo de *Junellia bryoides*, especie tipo del subgénero *Thryothamnus* sección *Junelliopsis* Botta (1989: 393) y se comprobó que presenta caracteres morfológicos propios del subgénero *Junellia*, por lo cual se transfiere la sección *Junelliopsis* a este subgénero. Se hace necesario entonces, realizar una

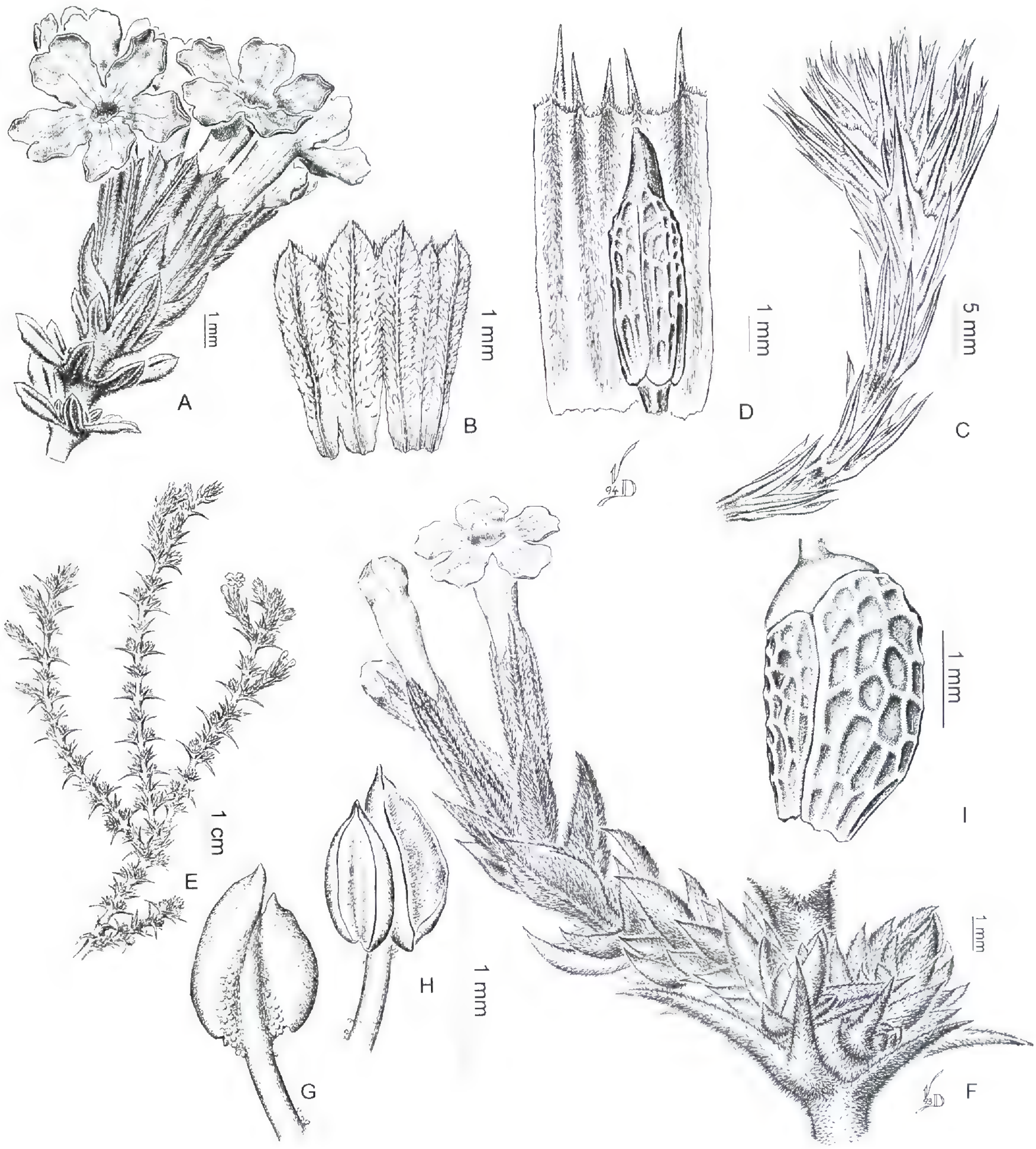


Figura 5. A, B. *Junellia bisulcata* var. *bisulcata*. —A. Inflorescencia. —B. Cáliz abierto. A, B de *Cabrera 31700*. C, D. *Junellia bisulcata* var. *campestris*. —C. Inflorescencia. —D. Cáliz abierto y fruto. C, D de *Krapovickas 7751*. E–I. *Junellia bryoides*. —E. Aspecto general. —F. Detalle de rama e inflorescencia. —G, H. Anteras. —I. Fruto. E–I de *Rhamer s.n.*, SGO 42472.

nueva combinación a nivel de sección para los taxones agrupados por Botta en la sección *Junellia* series *Erinaceae* y *Seriphioideae*. Aquí se considera que estas series no presentan caracteres suficientes para el mantenimiento de las mismas.

14. Junellia bisulcata (Hayek) Moldenke.

14a. Junellia bisulcata (Hayek) Moldenke var. **bisulcata**, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. Basónimo: *Verbena bisulcata* Hayek, Bot. Jahrb. 42: 164. 1909. TIPO: Bolivia. Puna Patanca, 3700 m, 1903–1904, *K. A. G. Fiebrig 2473* (lectotipo, aquí designado, K!; isotipos, BM!, K!, L no visto, P!, SI!, US no visto, W no visto, B† foto FM 17404 SI!). Figura 5A, B.

Arbustos bajos, postrados, muy ramosos, de ramas decumbentes, estriadas, de 10–40 cm, a veces erguidas, pubescencia híspida, pelos adpreso-retrorsos. Hojas dimorfas, las de los macroblastos de 3–6 (–7.5) × 1.5–5 mm, trisectas, lóbulos desiguales, angostamente oblongos, el central de mayor longitud,

rígidos, espinescentes, ápice mucronado, el nervio central prominente en el envés y margen ciliado entero, revoluto, pubescencia escasa, generalmente patentes; hojas de los braquiblastos 2.5–3 × 2.5–2.8 mm, glabras subcrasas, 3- o 2-lobadas, más raramente enteras, ápice obtuso hasta subagudo, por lo general brevemente mucronadas, si el braquiblasto se desarrolla aparecen hojas trisectas. Monobotrios en espigas contraídas de 1–1.5(–2.5) cm, plurifloras de 6 a 12 (o 18) flores; brácteas de 3–5 mm, enteras, ovadas, en general de ápice agudo, a veces cuspidado, cilioladas, iguales o más breves que el cáliz. Cáliz de 5–7 mm, con pelos breves, patentes, esparcidos en su superficie, más densos en su mitad superior, dientes del cáliz triangulares, menor a 1 mm; corola de 10–13 mm, lila, rosada o púrpura, glabra exteriormente, velloso en el interior, fauce pubescente; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6.5–8 mm, base del estilo ensanchada. Clusas de 4.5–5.5 mm, glabras, subcilíndricas, rostradas, cubierto por la base del estilo, cara dorsal reticulada, lisa en el ápice, castaño claro, caras comisurales blancas, papilosas.

Distribución geográfica. Sur de Bolivia y noroeste de Argentina desde las provincias de Jujuy a La Rioja. Fitogeográficamente crece en el dominio Andino-patagónico, provincia Puneña, entre los 2040 y 3300 m (Fig. 1B).

Fenología. Florece y fructifica de noviembre a marzo.

Iconografía. Botta, 1984: 344, fig. 5. Botta, 1993: 73, fig. 32.

Observaciones. Especie afín a *Junellia juniperina* (Lag.) Moldenke que presenta las clusas truncadas y los tallos con pubescencia híspida.

El holotipo de esta especie estaba depositado en B, y actualmente está destruido, razón por la cual se eligió un lectotipo entre los isotipos encontrados.

Material adicional examinado. ARGENTINA. **Catamarca**: *Jörgensen 1733* (SI). **Jujuy**: Dpto. Humahuaca, Chapuí Rodeo, nacientes del Arroyo La Cueva, *Novara 8770* (SI). **La Rioja**: Dpto. Chilecito, Cuesta de Miranda, *Halloy 955* (SI). **Salta**: Dpto. Cachi, Cachipampa, *Cabrera 30747* (SI). **Tucumán**: Dpto. Tafí, subida al Abra del Infiernillo desde Amaicha, *Kiesling 5855* (SI). BOLIVIA. **Tarija**: Méndez, Iscayachi, *Ehrich 48* (SI); Avilez, cerca de Cobre, *Bastián 602* (SI).

14b. Junellia bisulcata var. **campestris** (Griseb.) Botta, Hickenia 2: 127. 1995. Basónimo: *Verbena juniperina* var. *campestris* Griseb., Abh. Königl. Ges. Wiss. Göttingen 19: 193. 1874. *Junellia juniperina* var. *campestris* (Griseb.) Moldenke, Phytologia 44: 215. 1979. TIPO: Argentina. Córdoba: Chañar, N de la prov. de Córdoba, 29 nov. 1871, *P. Lorentz 7* (holotipo, GOET no visto; isotipos, CORD!, K!, SI!, K foto SI!). Figura 5C, D.

Difiere de la var. *bisulcata*, principalmente por las hojas en general no patentes y los dientes del cáliz atenuados, los anteriores ca. 1.5 mm long.

Distribución geográfica. Argentina, en las provincias de Córdoba y Santiago del Estero, en la provincia fitogeográfica Chaqueña, distrito Chaqueño-serrano (Fig. 1B).

Fenología. Florece y fructifica en verano.

Material adicional examinado. ARGENTINA. **Córdoba**: Dpto. Sobremonte, San Francisco de Chañar, *Burkart 29642* (SI). **Santiago del Estero**: Dpto. Ojo de Agua, ruta 9, límite con Córdoba, *Burkart 20110* (SI).

15. Junellia bryoides (Phil.) Moldenke, Lilloa 5: 394. 1940. Basónimo: *Verbena bryoides* Phil., Fl. Atac.: 40. 1860. TIPO: Chile. Desierto de Atacama: Alto de Varas, ene. 1854, *R. A. Philippi s.n., p.p.* SGO 54675 (lectotipo, designado por Acevedo de Vargas (1951: 53), SGO!). Figura 5E–I.

Arbustos erectos 1 m de alt.; tallos con pelos simples, patentes, macroblastos más o menos erectos. Hojas dimorfas, sésiles, las de los macroblastos 3-partidas o 3-sectas, lóbulos de 1.5–2.5 mm, oblongos, carnosos, ápice agudo, rígido, escabrosos en toda su superficie, pelos algo más largos en el margen, segmento medio de mayor longitud que los laterales; hojas de los braquiblastos de 1.5–2 mm, enteras, ovadas, carnosas, ápice rígido; pubescencia igual a las anteriores. Monobotrios plurifloros; brácteas de 3–3.5 mm, enteras, ovadas, ápice agudo, rígidas; con los pelos más largos que los de las hojas, muy aislados en la superficie, más abundantes en el margen. Cáliz de 3.5–4 mm, pubescente exteriormente; dientes desiguales, subulados, rígidos, el mayor 1 mm; corola de 10–10.5 mm, glabra en el exterior; fauce glabra o con escasísimos pelos semejantes a los del interior del tubo corolino; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 7–7.5 mm, base del estilo ensanchada. Clusas 2 mm, glabras, ápice truncado y cubierto por la base del estilo, cara dorsal pardo obscura, caras comisurales papilosas.

Nombre vulgar. "Cola de zorro", "pata de perdiz" (Acevedo de Vargas, 1951: 53).

Distribución geográfica. Chile, desierto de Atacama. Crece en el dominio Andino-patagónico, Provincia del Desierto, alrededor de los 3500 m (Fig. 1B).

Fenología. Florece y fructifica en verano.

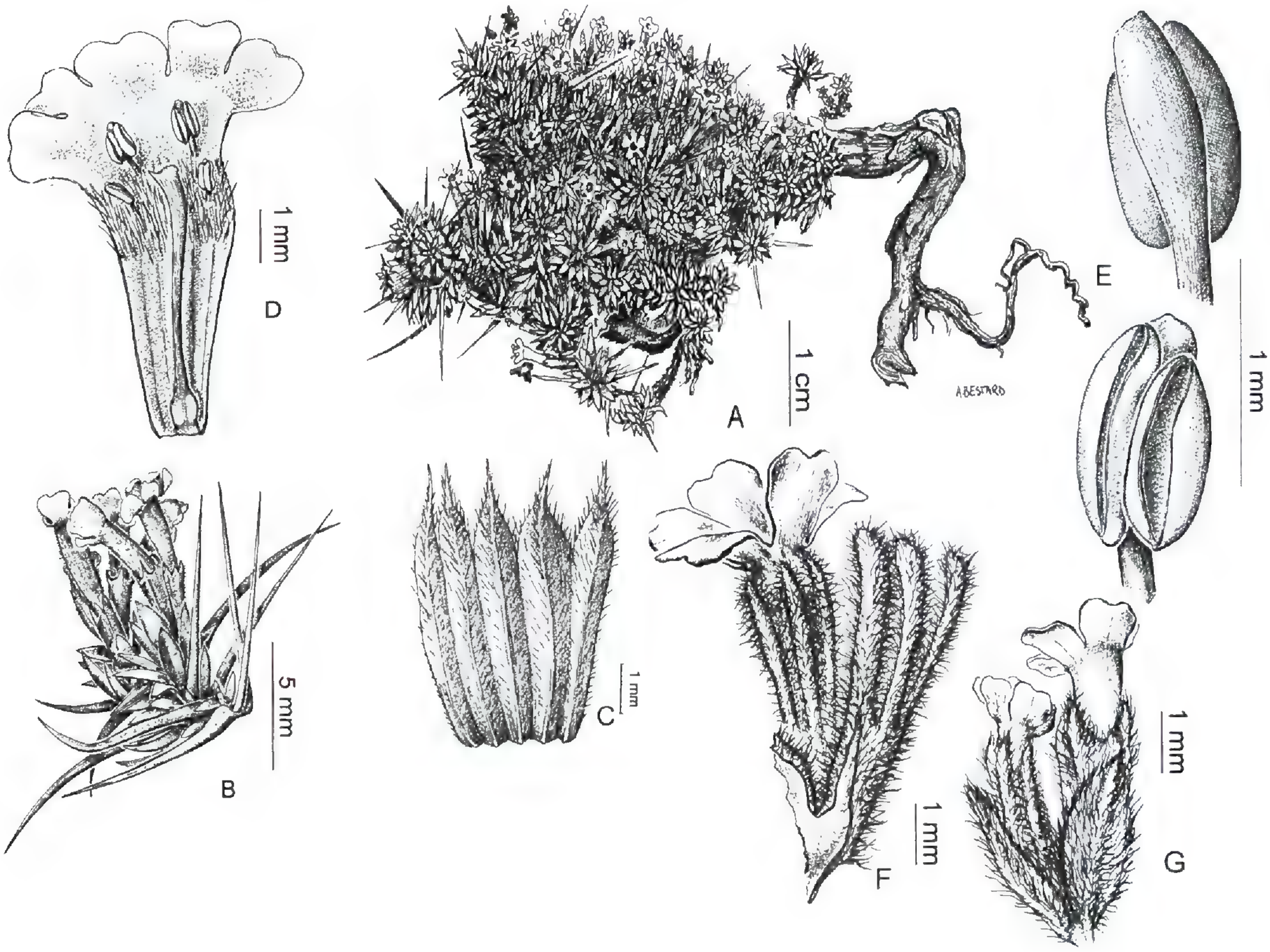


Figura 6. A–E. *Junellia caespitosa*. —A. Aspecto general. —B. Detalle de rama e inflorescencia. —C. Cáliz abierto. —D. Corola. —E. Anteras. A–E de *Múlgura 134*. —F. *Junellia digitata* var. *digitata*. Flor y brácteas (de *Cabrera 31856*). —G. *Junellia digitata* var. *integerrima*. Flor y bráctea (de *Cabrera 8041*).

Iconografía. Philippi, 1860: tab. V, fig. C, sub *Verbena bryoides*.

Observaciones. *Junellia bryoides* y *J. hystrix* son muy similares en su aspecto vegetativo, porte y desarrollo de la planta; esta última presenta la base del estilo incluido entre las clusas. Esta similitud entre las especies mencionadas, motivó la sinonimia propuesta por Moldenke (1940: 394).

Acevedo de Vargas (1951: 52) las reconoce como entidades independientes (bajo el género *Verbena*) basándose en las características de la pubescencia de las hojas y de la corola, haciendo constar que en el ejemplar SGO 54675 hay mezcla de las dos especies y elige al mismo como lectotipo de *V. bryoides*.

Botta (1989: 393) funda subgénero *Thryothamnus* sección *Junelliopsis*, y designa como especie tipo a *Junellia bryoides* sin mencionar a *J. hystrix*.

Material adicional examinado. CHILE. **I Región**: Tarapacá, Jaiña, *Rahmer s.n.*, SGO 42472 (SGO). s. loc., *Philippi s.n.* (BM).

16. Junellia caespitosa (Gillies & Hook. ex Hook.) Moldenke, Lilloa 5: 394. 1940. Basónimo: *Verbena caespitosa* Gillies & Hook. ex Hook., Bot. Misc. 1: 165. 1829. TIPO: Argentina. Mendoza: "Uspallata summit of the Uspallata range mountain", *J. Gillies s.n., Herb. Hook.* (lectotipo, designado por Botta (1989: 388), K!, K foto SI!). Figura 6A–E.

Verbena comberi Sandwith, Kew Bull. 1927: 185. 1927, syn. nov. *Junellia comberi* (Sandwith) Moldenke, Lilloa 5: 394. 1940. TIPO: Argentina. Neuquén: río Coyunco, 12 dic. 1925, *H. F. Comber 138* (holotipo, K!, K foto SI!; isotipos, BM!, TEX no visto).

Caméfitos pulvinados, en cojines hemisféricos densos o caméfitos en placas, macroblastos rastreros, bien desarrollados o no. Hojas dimorfas, la de los macroblastos de 5–15 × 0.5–2 mm, enteras, lineares, espiniformes; hojas de los braquiblastos de 1.5–2.5 × 1–1.5 mm, enteras, ovadas, carnosas, imbricadas, ambas con escasos pelos cortos, antrorsos en la cara abaxial y pelos largos adpresos densos en la cara adaxial. Monobotrios bifloros; brácteas 2.5–3(–5) × (1.5–)2–2.5 mm, ovadas o anchamente ovadas, ápice agudo, margen ciliado, pubescencia adpresa en ambas caras. Cáliz de 4.5–5(–6) mm, dientes desiguales, 1 mm, triangulares, no punzantes, con pelos cortos suaves y adpresos, sobre las costillas y los dientes, a

veces erectos en la parte basal del cáliz; corola 10–10.5 mm, lila claro, glabra en el exterior, pubescente en el interior, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6.5–7 mm, base del estilo ensanchada. Clusas de 2.5–3(–4) mm, glabras, ápice truncado, cubierto por la base del estilo, base angostada, cara dorsal reticulada, caras comisurales papilosas.

Nombre vulgar. "Llareta" (Hooker, 1829: 165).

Distribución geográfica. Chile, regiones III y IV y en Argentina en San Juan, Mendoza y Neuquén; en el extremo sur de la provincia fitogeográfica Puneña del dominio Andino-patagónico, al oeste de la provincia del Monte, dominio Chaqueño, y en la provincia Patagónica, dominio Alto-andino, entre los 1400 y 3700 m (Fig. 1B).

Fenología. Florece y fructifica en verano.

Iconografía. Sanzin, 1919: 108, fig. 11; Botta, 1999: 167, fig. 123 (sub *J. comberi*).

Observaciones. Esta especie es afín a *Junellia toninii* de la que se diferencia por ser un arbusto bajo con hojas enteras o partidas, monobotrios plurifloros y cáliz glabro o con pubescencia híspida.

Material adicional examinado. ARGENTINA. **Mendoza**: Dpto. Las Heras, Cruz del Paramillo, *Rotman 414* (SI). **Neuquén**: Dpto. Chos Malal, 21 km al N de Chos Malal, *Vallerini 3478* (SI). **San Juan**: Dpto. Calingasta, Sierra de El Tontal, *Kiesling 6558* (SI). CHILE. **III Región**: Atacama, Dpto. Vallenar, Laguna Grande, *Johnston 5937* (K). **IV Región**: Coquimbo, cordillera de Huanta, *Gay 310* (P).

17. **Junellia connatibracteata** (Kuntze) Moldenke, Lilloa 5: 394. 1940. Basónimo: *Verbena connatibracteata* Kuntze, Revis. Gen. Pl. 3(2): 255. 1893. TIPO: Argentina. Mendoza: Paso Cruz, arroyo de los Papagayos, 1650 m, ene. 1892, *O. Kuntze s.n.* (lectotipo, aquí designado, NY!).

Verbena dolichothyrsa Sandwith, Kew Bull. 1927: 186. 1927, syn. nov. *Junellia dolichothyrsa* (Sandwith) Moldenke, Lilloa 5: 395. 1940. TIPO: Argentina. Neuquén, Zapala: Zapala, Cerro Lotena and Coyunco, 900 m, 14 Oct. 1925, *H. F. Comber 79* (holotipo, K!, K foto SI!; isotipos, BM!, TEX no visto).

Arbustos bajos de 0.15–0.60 m de alt. Macroblastos erectos, hispídulos. Hojas dimorfas, las de los macroblastos de 5–10 × 1–1.5 mm, sésiles, connadas en la base, ovadas a ovado-angostas, de sección triqueta, ápice agudo, rígido punzante, en general recurvas, con el nervio medio prominente en la cara abaxial, con pelos cortos, adpresos y de densidad variable en ambas caras; hojas de los macroblastos de 3.5–4 mm, ovadas, carnosas, ápice agudo rígido, algo plegadas, pelos adpresos en la cara abaxial, margen ciliado. Monobotrios contraídos, plurifloros; brácteas de 3.5–4 mm, anchamente ovadas, connadas en la base, de ápice agudo, a veces largamente atenuado, con pubescencia adpresa. Cáliz de 5.5–7 mm, dientes triangulares con ápice atenuado y rígido, el adaxial muy breve; pubescencia de densidad variable, adpresa en las regiones intercostales, a veces algo híspida sobre los nervios; ciliado en el ápice, entre los dientes; corola de 10–13 mm, blanco crema hasta rosada, glabra en el exterior, fauce glabra y tubo pubescente en el interior; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6–6.5 mm, cilíndrico, base del estilo ensanchada. Clusas de 4 × 1.5 mm, glabras, cilíndricas, ápice truncado, cubierto por la base del estilo, cara dorsal castaña, rugosa, caras comisurales blancas, papilosas, papilas digitaliformes.

Nombre vulgar. "Tomillo" (Ruíz Leal, 1972: 240).

Distribución geográfica. Argentina en las provincias de Mendoza, La Pampa, suroeste de Buenos Aires, Neuquén, Río Negro y Chubut; fitogeográficamente habita en la provincia Patagónica en los distritos de la Payunia, Occidental y Central, a 900 m (Fig. 1C).

Fenología. Florece de octubre a febrero.

Iconografía. Sanzin, 1919: 123, fig. 27; Ruíz Leal, 1972: 239, lám. 74, fig. 275; Botta, 1999: 171, fig. 126.

Observaciones. La especie se caracteriza por sus brácteas connadas y hojas recurvadas, que la diferencian de las restantes de la sección.

En la descripción original se citan dos ejemplares de los cuales, se designa como lectotipo al ejemplar *Kuntze s.n.*, por ser el más completo.

El mayor o menor grado del desarrollo de los braquiblastos, así como la variación en la densidad de la pubescencia, otorgan aspectos muy variados a esta especie. Monticelli (1938: 358) teniendo en cuenta estos caracteres, consideró tres formas ecológicas, sin descripción en latín, ni cita de ejemplares, por lo tanto son nombres inválidos: (1) *Verbena connatibracteata* f. *normalis* Monticelli (Lilloa 3: 358. 1938); (2) *Verbena connatibracteata* f. *glomerata* Monticelli (Lilloa 3: 358. 1938); (3) *Verbena connatibracteata* f. *rosulata* Monticelli (Lilloa 3: 358. 1938) [*Junellia connatibracteata* (Kuntze) Moldenke f. *rosulata* (Monticelli) Moldenke (Phytologia 2: 135. 1946)].

En *Flora Patagónica* (Botta, 1999: 175), se consideró a *Junellia dolichothyrsa*, como sinónimo de *J. mulinoides*. El aspecto general, las hojas enteras

y las brácteas connadas han sido los caracteres considerados para sinonimizar *J. dolichothyrsa* con *J. connatibracteata*.

Material adicional examinado. ARGENTINA. Patagonia 50/3, 1882/4, *Kuntze s.n.* (paratipo NY, isoparatipos LP, SI). **Buenos Aires**: Partido Villarino, *Fernández y Zalba s.n.*, Herb. Villamil 7159 (BBB). **Chubut**: Dpto. Paso de los Indios, ruta 25, a 42 km S de Paso de Indios, *S. Arroyo 66* (SI). **La Pampa**: Dpto. Limay Mahuida, Puesto Garro, *Steibel 2486* (SI). **Mendoza**: Dpto. Luján de Cuyo, 100 km S of Mendoza, *Bocher & Hjerting 672* (SI). **Neuquén**: Dpto. Catan Lil, a 4 km de Las Cortaderas hacia Charahuilla, *Correa 7901* (SI). **Río Negro**: Dpto. A. Alsina, ruta 3, Km 1107, entre Viedma y San Antonio Oeste, *Correa 2457* (SI).

18. Junellia erinacea (Gillies & Hook. ex Hook.) Moldenke, Lilloa 5: 396. 1940. Basónimo: *Verbena erinacea* Gillies & Hook. ex Hook., Bot. Misc. 1: 164. 1829. TIPO: Argentina. Mendoza: Portezuelo de Uspallata, "a low shrub with flowers growing near community along with G. C. h.", 29 ene. 1824, *J. Gillies s.n.* (lectotipo, designado por Botta (1989: 386), K!, K foto SI!; isotipo, BM!).

Caméfitos pulvinados, cojines hemisféricos, espinosos, no compactos. Ramas de 7–12 cm. Hojas homomorfas, de 10–20 × 1–1.5 mm, lineares, espiniformes, connadas en la base, margen piloso, híspidas en la cara abaxial y con escasos pelos casi adpresos en la adaxial, opuestas en macroblastos e imbricadas en braquiblastos. Monobotrios paucifloros, generalmente de 2 o 3 flores, brácteas de 4–6 × 3–4 mm, connadas en la base, ovado-anchas, ápice subulado, rígido, con el margen piloso y la superficie híspida y glandulosa. Cáliz de 5.5–6.5 mm, híspido, glanduloso, dientes subulados, espinescentes, el mayor de 1.5 mm, el adaxial muy breve; corola de 10.5–11.5 mm, lila o blanca; en el exterior glabro y piloso en el interior; fauce glabra o con escasos pelos y piloso en el interior; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 8–8.5 mm, base del estilo ensanchada. Clusas de 4.5–5 mm, glabras, ápice brevemente rostrado y cubierto por la base del estilo, base angostada, cara dorsal reticulada, caras comisurales lisas.

Distribución geográfica. Argentina en las provincias de La Rioja, San Juan, Mendoza y Río Negro; fitogeográficamente crece en el límite de la provincia del Monte, con la provincia Alto-andina, y la Patagónica, en general entre los 2500 y 3000 m, en suelos rocosos, generalmente sueltos (Fig. 1D). Fue citada para San Luis, por Minoprio et al. (1967: 26) pero dado que no hay registros de herbario que avalen esta distribución, no se ha considerado que la especie este presente en la provincia de San Luis.

Fenología. Florece y fructifica en verano.

Iconografía. Hooker, 1829: fig. 48; Sanzin, 1919: 108, fig. 12 (sub *Verbena erinacea*); Botta, 1989: 385, fig. 5.

Observaciones. Especie afín a *Junellia caespitosa* de la que se diferencia por tener las hojas homomorfas espiniformes y las clusas brevemente rostradas.

Material adicional examinado. ARGENTINA. **La Rioja**: Dpto. La Madrid, entre Lampaya y río Las Cuevas, *Kraporickas 5563* (BAB). **Mendoza**: Dpto. Las Heras, Paramillo de Uspallata, *Ruíz Leal 11140* (AUS, MERL). **Río Negro**: Dpto. Valcheta, Meseta de Somuncurá, base del Cerro Corona, 10 ene. 2002, *Bártoli s.n.*, BAA 24787 (SI). **San Juan**: Dpto. Calingasta, El Leoncito, Observatorio, *Kiesling 6498* (SI).

19. Junellia juniperina (Lag.) Moldenke, Lilloa 5: 396. 1940. Basónimo: *Verbena juniperina* Lag., Gen. Sp. Pl. 19. 1816. TIPO: Argentina. Mendoza: San Carlos, camino a Laguna Diamante, 2000 m, 2 feb. 1950, *A. Soriano 4057* (neotipo, aquí designado, SI!).

Verbena juniperina var. *grisea* I. M. Johnst., Contr. Gray Herb. 81: 98. 1928. *Junellia juniperina* var. *grisea* (I. M. Johnst.) Moldenke, Lilloa 5: 397. 1940. TIPO: Perú. Arequipa: ravines, S slope of Chachani, N of Arequipa, 3355 m, Mar. 1920, *Hinckley 76* (holotipo, GH no visto; isotipos, NY!, US no visto).

Arbustos erguidos o postrados. Macroblastos erguidos, a veces decumbentes, tallos con pubescencia generalmente híspida, variable en densidad. Hojas dimorfas, las de los macroblastos, de 4–5 × 2.5–3 mm, profundamente 3-sectas, connadas, segmentos de 5–6.5 × 1 mm, angostamente elípticos o lineares, rígidos, pungentes, subiguales, nervio central prominente en la cara interior y margenes engrosados; hojas de los braquiblastos enteras, 2-lobadas o 2–3-sectas, más pequeñas que las anteriores, pubescencia con pelos simples y glandulares. Monobotrios en espigas alargadas, de 2–3 cm, plurifloras, de 30 flores; brácteas de 5–7.5 × 1.5–2 mm, enteras, ovales, pungentes, pubescentes y con pelos largos y rígidos en el margen. Cáliz de 6.5–8 mm, dientes de 0.5–1 mm, subiguales, pungentes, pelos simples y glandulares; corola lila de 10–12.5 mm, glabra en el exterior, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6.5–8 mm, base del estilo ensanchada. Clusas de 3.5–4 mm, glabras, ápice truncado, a veces brevemente rostrado y cubierto por la base del estilo, cara dorsal reticulada, castaño rojizo, caras comisurales casi planas, papilosas, blanquecinas.

Cariología. $n = 10$ (*Junellia juniperina*, Poggio et al., 1988: 543).

Nombres vulgares. "Monte de la yegua" (Ruíz Leal, 1972: 168), "diablo fuerte" (de la Peña & Pensiero, 2004: 68), "heliotropo de la sierra" (Sanzin, 1919: 113), "cola de león" (del ejemplar *Meyer 8269*, LIL).

Usos. Según Roig (2001) es medicinal, depurativa de la sangre.

Distribución geográfica. Sur de Perú, sur de Bolivia, norte y centro de Chile y noroeste de Argentina; en la provincia fitogeográfica Altoandina y Puneña; crece entre los 1900 y 3600 m (Fig. 1D).

Iconografía. Sanzin, 1919: 114, fig. 17; Ruíz Leal, 1972: 169, lám. 52, fig. 189; Botta, 1984: 340, fig. 4 (todos sub *Verbena juniperina*).

Observaciones. Especie similar a *Junellia bisulcata* de la que se diferencia por el ápice de las clusas rostrado y la pubescencia de los tallos adpresa.

En este caso para neotipificar el taxón se eligió un nuevo ejemplar que reúne las características de la especie.

Verbena juniperina Gillies & Hook. ex Hook. (Bot. Misc. 1. 163. 1829) es ilegítimo por homónimo posterior y de la observación del material tipo se comprobó dicha especie es un sinónimo taxonómico.

El ejemplar *Kurtz 6788*, citado por Moldenke (1940: 397) para la provincia de Córdoba, corresponde a *Junellia bisulcata* var. *campestris*. Además Moldenke (1940: 397) expresa: "originally distributed as *Verbena juniperina* var. *charoides* Hieron."; este nombre no se ha registrado en ninguna publicación, por lo tanto se debe considerar como nom. nud. Por último, Moldenke (1980: 186) cita la especie para Chubut, sin mencionar ningún ejemplar; probablemente se trate de *J. ulicina*.

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Andalgalá, along roadside betw. Andalgalá & Minas Capillitas, *Cantino 431* (SI). **La Rioja**: Dpto. Chilecito, Quebrada Los Manzanos, *Okada 2719* (SI). **Mendoza**: Dpto. Las Heras, Caracoles de Villavicencio, *Rotman 395* (SI). **Salta**: Dpto. Cafayate, de Salta a Cafayate, *Gamerro s.n.*, SI 26244 (SI). **San Juan**: Dpto. Sarmiento, quebrada de La Flecha y quebrada el Río de los Sombreros, *Kiesling 4160* (SI). **Tucumán**: Dpto. Tafí, Cuesta del Infiernillo, Km 84, *Burkart 22071* (SI). BOLIVIA. **Tarija**: Aviles, Yunchara, *Meyer 21561* (LIL). CHILE. **I Región**: Arica, Altos de Putre, *Escobar X 112* (SI). **Región Metropolitana**: Plata, entre Santaigo & Cordova, *Gay s.n.* (P). PERÚ. **Arequipa**: pampa de los arrieros, *Stafford 723* (K). **Ayacucho**: Coracora, Parinacochas, *Cerrate 4859* (SI). **Huancavelica**: Castrovirreina, near Córdova, *Goodspeed 30268* (LIL). **Moquegua**: Mariscal Nieto, *Dillon 3336* (F). **Tacna**: Tarata, camino a Caro, *La Torre 1837* (UMS).

20. Junellia odonellii Moldenke, Phytologia 2(11): 466. 1948. TIPO: Argentina. Santa Cruz, Güer Aike: Río Gallegos, s.f., *C. A. O'Donell 4000* (holotipo, NY!; isotipos, P no visto, SI!, P foto SI!).

Caméfitos pulvinados, tallos glabros o escasamente híspidos, macroblastos de 6–12 cm, erectos a decumbentes. Hojas dimorfas, las de los macroblastos de 4–7 mm, opuestas, subsésiles, profundamente 3-partidas, lóbulos lineales, con ápice agudo, punzante, los laterales algo más breves, ciliados en el margen, con pubescencia antrorsa; hojas de los braquiblastos tripartidas, carnosas, glabras, de menor tamaño que las de los macroblastos. Monobotrios de 3 a 10 flores en el ápice de braquiblastos más desarrollados; brácteas de 4.5–7 mm, 3-partidas, similares a las hojas del macroblasto. Cáliz de 5–6 mm, con pubescencia híspida, rala; dientes triangulares, desiguales, breves, de ápice atenuado, rígido; corola de 12–14 mm, blanca, pubescente-glandulosa en el exterior; tubo pubescente, en el interior, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 8.5–9 mm, base del estilo ensanchada. Clusas de 4 × 0.8 mm, glabras, ápice truncado y cubierto por la base del estilo, base angostada, cara dorsal reticulada.

Distribución geográfica. Se la encuentra desde Neuquén a Santa Cruz, en Argentina y en el sur de Chile región XII, formando carpetas leñosas entre rocas, en cumbres de cerros; en la provincia fitogeográfica Patagónica (Fig. 1E).

Fenología. Florece y fructifica en primavera y verano.

Iconografía. Botta, 1999: 175, 180, fig. 131.

Observaciones. Verbena odonellii (Moldenke) Tronc. (Darwiniana 18: 313. 1974), esta combinación realizada por Troncoso, es ilegítima por omitir el lugar de publicación del nombre original de acuerdo con el Art. 33.3 del *Código Internacional de Nomenclatura Botánica* (Greuter et al., 2000).

Especie afín a *Junellia ulicina* de la que se diferencia por el mayor tamaño y el ápice acuminado de los dientes del cáliz, los monobotrios 2- o 3-floros y el mayor tamaño de la bráctea.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Escalante, Pampa del Castillo, punto panorámico, *Cabrera 33253* (SI). **Neuquén**: Dpto. Los Lagos, Ea. Fortín Chacabuco, co. Lepún, *Boelcke 7582* (BAB). **Santa Cruz**: Dpto. Güer Aike, ruta 40, de Güer Aike a La Esperanza, 72 km al W de Río Gallegos, *Botta 507* (SI). CHILE. **XII Región**: Magallanes, Estancia La Portada, *Dollenz 1368* (CONC).

21. Junellia seriphioides (Gillies & Hook. ex Hook.) Moldenke, Lilloa 5: 400. 1940. Basónimo: *Verbena seriphioides* Gillies & Hook. ex Hook., Bot. Misc. 1: 164. 1829. TIPO: Argentina. Mendoza: betw. Río Tunuyán & Río Mendoza, also in S side of latter river, Oct. 27–Nov. 5,

1824, *J. Gillies s.n.* (lectotipo, designado por Botta (1984: 334), K!, K foto SI!; isotipo, BM!, BM foto SI!). Figura 3H, I.

Verbena echinata Phil., Anales Univ. Chile 36: 191. 1870. TIPO: Argentina. Mendoza: Andes mendocinum, 1868–1869, *R. Philippi s.n.*, SGO 42479 (lectotipo, designado por Acevedo de Vargas (1951: 65), SGO!; isotipos, K!, P!).

Verbena seriphioides var. *lanigera* Hieron., Bol. Acad. Ci. (Córdoba) 3: 370. 1880. TIPO: Argentina. Santa Cruz: en el campo cerca del Río Santa Cruz, 10 oct. 1874, *C. Berg 152* (holotipo, CORD no visto).

Verbena briquetii J. F. Macbr., Candollea 5: 402. 1934. Nombre reemplazado: *Verbena hystrix* Briq., Annuaire Conserv. Jard. Bot. Genève 4: 15. 1900, nom. illeg., non *V. hystrix* Phil., Anales Univ. Chile 90: 610. 1895. TIPO: Argentina. Mendoza: Pampa de San Rafael, s.f., *E. Wilczek 45* (holotipo, G!, G foto FM 24687 SI!; isotipos, K!, SI!, K foto SI!).

Junellia seriphioides var. *tomentosa* Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. TIPO: Argentina. Mendoza: near Mendoza, Oct. 1896, col. Bodenbender, herb. *F. Kurtz 10012* (holotipo, NY no visto; isotipos, CORD no visto, SI!).

Junellia seriphioides var. *glabra* Moldenke, Phytologia 2: 409. 1948. TIPO: Argentina. Chubut: Puerto Madryn, 24 oct. 1945, *C. O'Donell 3240* (holotipo, S no visto, S foto SI!; isotipos, NY no visto, SI!).

Arbustos de 0.5–0.8 m alt. Macroblastos erectos, ramificados, a veces con raíces adventicias en la base, los más jóvenes pubescentes, al envejecer exfoliables en lonjas o láminas alargadas, braquiblastos de 2–2.5 mm, muy ramificados. Hojas dimorfas, las de los macroblastos de 5–7 × 1–1.5 mm, enteras, rara vez 3-sectas (en este caso los lóbulos laterales apenas desarrollados), espiniformes, connadas en la base, cuando jóvenes pubescentes en ambas caras, hojas de los braquiblastos de 1 × 1 mm, enteras, carnosas, tetrásticas, imbricadas, lámina ovada, la mitad superior engrosada, bordes incurvos formando un surco tapizado por pelos suaves, adpresos y antrorsos, margen ciliolado. Monobotrios paucifloros, de 2 a 3 flores en el ápice de los braquiblastos; brácteas de 2.5 mm, ovadas, levemente pubescentes en la cara adaxial. Cáliz de 3.5–4 mm, externamente velloso (a veces glabro), y con pelos glandulares breves, hacia el ápice la pubescencia adpresa; corola de 10–12 mm, blanca o lila-pálido, glabra exteriormente; tubo recto, velloso en el interior; fauce glabra o con escasos pelos; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 4–5 mm, base del estilo ensanchada. Clusas de 3.5 mm, glabras, brevemente aladas, ápice truncado y cubierto por la base del estilo, cara dorsal reticulada, pardas, caras comisurales papilosas, blanquecinas.

Cariología. $n = 10$ (*Junellia seriphioides*, Schnack & Covas, 1947: 5).

Nombre vulgar. "Tomillo macho" (Sanzin, 1919: 87; Ruíz Leal, 1972: 242), "perlilla", "perlita", "rosita" (Cabrera, 1957: 398), "rosa de la puna" (Moldenke & Moldenke, 1949: 7), "rosa de cordillera" (Muñoz Pizarro, 1966: 150).

Usos. En Salta "los nativos lo usan como refrescante cerebral para cuyo efecto se lavan la cabeza con dicha infusión", del ejemplar BA 27/891.

Distribución geográfica. Bolivia, Chile y Argentina, desde el noroeste hasta Patagonia. Crece en suelos arenosos y pedregosos en las provincias Puneña y Patagónica del Dominio Andino-patagónico hasta los 4200 m y en la provincia del Monte del Dominio Chaqueño (Fig. 2A).

Fenología. Florece y fructifica en primavera y verano.

Iconografía. Sanzin, 1919: 110, fig. 14 (sub *Verbena seriphioides*); Cabrera, 1957: fig. 1; Morello, 1958: 118, fig. 50; Roig, 1970: 143, lám. 70 (sub *V. seriphioides*); Ruíz Leal, 1972: 241, lám. 75, fig. 282 (sub *V. seriphioides*); Botta, 1984: 333, fig. 1 (sub *V. seriphioides*); Botta, 1993: 68, fig. 29; Botta, 1999: 183, fig. 134.

Observaciones. Esta especie se caracteriza por sus ramas rígidas, sus hojas punzantes y sus inflorescencias paucifloras. Es muy afín a *Junellia spissa*, de la que se diferencia por la presencia de clusas no aladas.

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Antofagasta de la Sierra, Antofagasta de la Sierra, *Ulibarri 747* (SI). **Chubut**: Dpto. Ameghino, 9 km N de Uzcudum, Ruta 31, *Correa 10212* (BAB, SI). **Jujuy**: Dpto. Cochinoca, Mina Aguilar, camino a la escuela del Río grande de la Poma, *Ancibor 31* (BAB, SI). **La Pampa**: Dpto. Caleu Caleu, barrancas and flat upland above comisaría de Río Colorado, *Bartlett 19935* (SI). **La Rioja**: Dpto. Gral. Sarmiento, quebrada del Río Peñón, *J. H. Hunziker* 2128 (SI). **Mendoza**: Dpto. San Carlos, Pareditas, La Jaula, cerros vecinos a la Agrupación de Gendarmería Nacional, *Ambrosetti s.n.*, Herb. Ruíz Leal 34616 (MERL). **Neuquén**: Dpto. Confluencia, Plottier, *Fortunato 90* (BAB). **Río Negro**: Dpto. Avellaneda, La Japonesa (Gob. Duval), *Krapovickas 22393* (SI). **Salta**: Dpto. La Poma, ruta 40, 55 km de San Antonio de los Cobres, 5 km antes del límite con Jujuy, *Cialdella 45* (SI). **San Juan**: Dpto. Angaco, Sierra de Pie de Palo, al Este de Mogote de los Corralitos, *Kiesling 4774* (SI). **San Luis**: Dpto. La Capital, Estancia San Martín del Alto Negro, *Anderson 1664* (SI). BOLIVIA. **Oruro**: L. Cabrera, de Salinas de G. Mendoza hacia el W, vía Iswaya, *Beck 11795* (SI). **Potosí**: Telamaya, *Cárdenas 48* (SI). CHILE. **I Región**: Iquique, Quebrada de Chusmiza, *Villagran 9072* (CONC). **II Región**: Antofagasta, Calama, Caspana, *Olivares 9621* (SI).

22. Junellia spissa (Sandwith) Moldenke, Lilloa 5: 401. 1940. Basónimo: *Verbena spissa* Sandwith, Kew Bull. 1927: 187. 1927. TIPO: Argentina. Neuquén, Zapala: Zapala, co. Lotena, 14 Oct. 1925, *H. F. Comber 78* (holotipo, K!, K foto SI!).

Caméfitos hasta 30 cm alt. Macroblastos muy ramificados desde la base, sinuosos, muy apretados; braquiblastos de 2–2.5 mm, arrosetados. Hojas dimorfas, las de los macroblastos de 4–7 mm, lineares, espinosas, híspidas en ambas caras, las de los braquiblastos de 1 mm, tetrásicas, sésiles, angostamente ovadas, agudas, carnosas, con pelos cortos antrorsos en ambas caras. Monobotrios de 2 flores en el ápice de los braquiblastos; brácteas ca. 2 × 3 mm, anchamente ovadas, ápice agudo y rígido, connadas en la base, híspidas en la cara exterior, ciliadas en el margen. Cáliz de 3.5–4 mm, híspido, dientes ca. 0.5 mm, triangulares, no pungentes, agudos; corola de 8.5–9 mm, lila o blanca, tubo recto, glabra exteriormente, pubescente interiormente; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 3.5–4.5 mm, base del estilo ensanchada. Clusas de 3–3.2 mm, glabras, ápice truncado y cubierto por la base del estilo, cara dorsal reticulada, caras comisurales papilosas, no aladas.

Distribución geográfica. Ha sido coleccionada desde Neuquén y Chubut, en suelos aridísimos, en la provincia fitogeográfica Patagónica (Fig. 2C).

Fenología. Florece entre noviembre y febrero.

Iconografía. Botta, 1999: 187, fig. 137.

Observaciones. Especie muy afín a *Junellia connatibracteata* de la que se diferencia por el número de flores en la espiguilla, siempre más de cinco flores. A veces parece un ejemplar muy achaparrado de *J. seriphioides* de la que se distingue por tener el fruto alado.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Florentino Ameghino, Ameghino, *Ruíz Leal 25670* (SI). **Neuquén**: Dpto. Picunches, bajada del Aorio, *Vallerini 3388* (SI); Dpto. Zapala, camino Neuquén–Zapala, bosque petrificado, Ruta 22, *Botta s.n.* (SI 26674).

23. Junellia toninii (Kuntze) Moldenke.

23a. Junellia toninii (Kuntze) Moldenke var. **toninii**, Phytologia 21: 253. 1971. Basónimo: *Verbena toninii* Kuntze, Revis. Gen. Pl. 3(2): 258. 1898. TIPO: Argentina. Patagonia, 1882–1884. *T. Moreno & Tonini s.n.* (holotipo, NY!; isotipos, LP!, SI!). Figura 4A–D.

Arbustos bajos, intrincados hasta 40 cm de alt., o caméfitos pulvinados hemisféricos ca. 15 cm alt., muy espinosos. Ramas erectas, densamente foliosas, de 10 20 cm. Hojas dimorfas, las de los macroblastos espiniformes, generalmente enteras, 4–6(–10) × 1–1.5 mm, lineares, a veces trisectas, lóbulos lineares, lóbulo medio ca. 5.5 × 1.5 mm, lóbulos laterales de 3.5 × 1 mm, o ambas formas en la misma rama, todas sésiles, agudas, ciliadas; hojas de los braquiblastos 1.5–2 × 0.5–1 mm, enteras, lineares, sésiles, ciliadas, ápice no punzante, carnosas o membranosas. Monobotrios en espigas de 3, 5 hasta 10 flores, en el ápice de macroblastos; brácteas de 3–6 × 1.5 mm, ovadas o angostamente ovadas, agudas o acuminadas, pilosas. Cáliz de 6.5–7 mm, pubérulo a pubescente, dientes de 1.5 mm, el adaxial muy breve, ciliado en los bordes; corola de 10–16.5 mm, blanca, azulada o lila, glabra exteriormente, pubescente interiormente en la mitad basal, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 4.5 mm, base del estilo ensanchada. Clusas de 4 mm, glabras, ápice truncado y cubierto por la base del estilo, cara dorsal reticulado-estriada, caras comisurales blancas, papilosas.

Distribución geográfica. Desde sur de Mendoza, hasta Santa Cruz, desde el nivel del mar hasta la serranías de la precordillera, en la provincia fitogeográfica Patagónica (Fig. 2D).

Fenología. Florece y fructifica en verano.

Iconografía. Botta, 1999: 191, fig. 140.

Observaciones. Se diferencia de la variedad *mulinoides* por la pubescencia del cáliz. Además es afín a *Junellia connatibracteata* de la que se diferencia por las brácteas connadas y las hojas de los macroblastos recurvas.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Escalante, ruta Nac. 3, 31 km S de Garayalde, *Botta 464* (SI). **Mendoza**: Dpto. Malargüe, camino a Valenciana, *Cabrera 33469* (SI). **Neuquén**: Dpto. Catán Lil, 15 km de Las Cortaderas hacia Charahuilla, *Corea 7911* (SI). **Río Negro**: Dpto. Ñorquinco, Choique, *Cabrera 33043* (SI). **Santa Cruz**: Dpto. Deseado, ruta Prov. 281, 52 km al W de Puerto Deseado, *Botta 478* (SI).

23b. Junellia toninii var. **mulinoides** (Speg.) P. Peralta & Múlgura, comb. nov. Basónimo: *Verbena mulinoides* Speg., Anales Soc. Ci. Argent. 53–54: 247. 1902. *Junellia mulinoides* (Speg.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. TIPO: Argentina. "In aridis maritimis Caleta Porfirio", mayo 1899, *Lahille s.n.* (lectotipo, designado por Botta (1989: 388), LP!). Figura 4E, F.

Se diferencia de la variedad típica, por presentar el cáliz glabro, apenas ciliado en los bordes y las brácteas glabras.

Distribución geográfica. Se la encuentra en terrenos áridos y laderas pedregosas desde Neuquén a Santa Cruz, en la provincia fitogeográfica Patagónica (Fig. 2D).

Iconografía. Botta, 1999: 177, fig. 130 sub *Junellia mulinoides*.

Fenología. Florece y fructifica en verano.

Observaciones. El lectotipo de *Verbena mulinoides* fue designado por Botta (1989: 388), como se expresa más arriba, pero en los manuscritos posteriores de la autora aparece el ejemplar de Illín, con anotaciones que consideran al mismo como lectotipo. Probablemente esto llevó a confusión en la citación del tipo en la *Flora Patagónica* (Botta, 1999: 175; "Chubut. dpto. Futaleufú, *N. Illín s.n.*, Mar. 1900, Holotypus LP 10941"). Por otra parte, en la etiqueta original del citado ejemplar, dice: Chubut: Carreuleufú, Patag., *N. Illin*, Mar. 1900, LPS 10401.

Acerca de los sinónimos de esta variedad ver *Junellia connatibracteata*.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Biedma, 8 km S de Madryn, ruta 1, *Correa 10160* (SI). **Neuquén**: Dpto. Añelo, Sierra Auca Mahuida, *Fabris 876* (SI). **Río Negro**: Dpto. San Antonio, Camino a Las Grutas, desvío al mar, *Botta 406* (SI). **Santa Cruz**: Dpto. Corpen Aike, a 70 km S Gobernador Gregores, camino a La Julia, *Boelcke 16255* (SI).

24. Junellia ulicina (Phil.) Moldenke, Lilloa 5: 402. 1940. Basónimo: *Verbena ulicina* Phil., Anales Univ. Chile 90: 611. 1895. TIPO: Chile. Colchagua: Valle Hermoso, ene. 1872, s. col., SGO 54682 (lectotipo, designado por Botta (1989: 386), SGO!, SGO foto SI!).

Verbena ameghinoi Speg., Revista Fac. Agron. Univ. Nac. La Plata 3: 61. 1897. *Junellia ameghinoi* (Speg.) Moldenke, Lilloa 5: 392. 1940. TIPO: Argentina. Santa Cruz: golfo de San Jorge, feb. 1896, *C. Ameghino s.n.* (holotipo, LP!, LP foto SI!).

Verbena intrincata Briq., Annuaire Conserv. Jard. Bot. Genève 4: 16. 1900. *Junellia intrincata* (Briq.) Moldenke, Lilloa 8: 416. 1942. TIPO: Argentina. Mendoza, San Rafael: Río Tordillos, mina de las Choicas, feb. 1897, *E. Wilczec 48* (holotipo, LAU no visto; isotipos, K!, P!, SI!, K foto SI!, G foto FM 24689 SI!).

Caméfitos pulvinados, pubescentes, ramas glabras o escasamente híspidas. Hojas dimorfas, las de los macroblastos tripartidas, espiniformes, de 5–10 mm (las basales menores que las superiores), lóbulo medio 2 mm mayor que los laterales, escabrosas; hojas de los braquiblastos no espiniformes, tripartidas, de 2 mm, carnosas, lóbulo medio mayor, superficie adaxial escabrosa. Monobotrios en espigas, de 2 a 3 flores, brácteas tripartidas, de 11.5–12 mm, similares a las hojas de los macroblastos. Cáliz de 6–7 mm, híspido-glanduloso, dientes ca. 1.5 mm, con ápice acuminado, rígido; corola de 12–15 mm, tubo recto, exteriormente glanduloso; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 12 mm, base del estilo ensanchada. Clusas de 4.5 mm, subcilíndricas, ápice obtuso y cubierto por la base del estilo, cara dorsal reticulada en la mitad superior, caras comisurales suavemente papilosas.

Distribución geográfica. Chile y Argentina, al sur del paralelo 32°, en las provincias de Mendoza, Neuquén, Chubut, Santa Cruz, desde el nivel del mar hasta la zona cordillerana a 1750 m (Fig. 2F).

Fenología. Florece en verano.

Iconografía. Botta, 1999: 199, fig. 143.

Observaciones. Es muy afín a *Junellia odonellii*: ambas especies son cojines, espiniformes, presentando las brácteas florales tripartidas. *Junellia odonellii* tiene monobotrios de 3- a 10-floros, brácteas menores y dientes del cáliz de 0.5 mm, atenuados.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Escalante, ruta Nac. 3, 76 km al S de Garayalde, *Botta 465* (SI). **Mendoza**: Dpto. Malargüe, Valle del Salado, Alto de Los Caracoles, camino del Sol, Km 13–14, *H. A. Lagiglia 6878* (SI). **Neuquén**: Dpto. Ñorquín, Cerro Huaile, ladera SE, *Biganzoli 1198* (SI). **Santa Cruz**: Dpto. Magallanes, Tres Cerros, Ea. La Lomita, pie del cerro, cerca de 3 km al E, ruta 3, *Boelcke 15368* (SI). CHILE. **VI Región**: Valle Hermoso, s. col., SGO 54682 (SGO, SI).

Junellia subgén. **Thryothamnus** Botta, Darwiniana 29: 390. 1989. TIPO: *Thryothamnus junciformis* Phil.

Arbustos de desarrollo variable, con hojas normales o reducidas; monobotrios o dibotrios plurifloros en espigas contraídas, cilíndricas; fauce de la corola glabra o pubescente; conectivo superando o no la longitud de las tecas, con o sin apéndices conectivales; base del estilo no ensanchada y más o menos hundida entre las clusas. Número cromosómico $x = 10$.

Junellia subgén. **Thryothamnus** secc. **Thryothamnus** Botta, Darwiniana 29: 391. 1989.

Verbena secc. *Verbenaca* ser. *Junciformis* Briq., p.p., in Engler & Prantl, Nat. Pflanzenfam. 4(3A): 147. 1895.

Verbena secc. *Junellia* ser. *Junciformis* (Briq.) Tronc., Darwiniana 18: 313. 1974.

Arbustos con ramas de sección poligonal o circular; hojas desarrolladas, enteras, no espiniformes; monobotrios o dibotrios plurifloros, en espigas contraídas; fauce de la corola glabra, estambres con conectivo de menor longitud que las tecas y sin apéndices conectivales.

En este caso las especies consideradas en esta sección coinciden con el tratamiento dado por Botta (1989: 391).

25. Junellia lavandulaefolia (Phil.) Moldenke, Lilloa 5: 397. 1940. Basónimo: *Verbena lavandulaefolia* Phil., Anales Univ. Chile 43: 521. 1873. TIPO: Chile. Las Damás, Río Teno, 1872, s. col., SGO 54719 (lectotipo, designado por Acevedo de Vargas (1951: 57), SGO!, SGO foto SI!; isotipo, SI!). Figura 7E.

Verbena colchaguensis Phil., Anales Univ. Chile 43: 521. 1873, syn. nov. *Verbena lavandulaefolia* Phil. var. *colchaguensis* (Phil.) Reiche, Fl. Chile 5: 281. 1910. *Junellia lavandulaefolia* var. *colchaguensis* (Phil.) Moldenke, Phytologia 2: 235. 1946. TIPO: Chile. Valle de las Damas, 1872, s. col., SGO 54708 (holotipo, SGO!).

Arbustos glabros, ramas erectas, estriadas de sección circular, entrenudos de 2–3 cm, generalmente sin desarrollo de braquiblastos. Hojas de 5–15 × 1–1.5 mm, generalmente todas enteras, a veces algunas hojas trífidas, lineares, sésiles, margen revoluto, base ensanchada y a veces persistente, pubescencia escabrosa escasa, pelos adpresos. Monobotrios en espigas, plurifloras, alargados a la madurez ca. 2 cm; brácteas de 3–5 × 2 mm, enteras, todas similares, angostamente elípticas, acuminadas, márgenes membranosos y ciliados, cara abaxial escabrosa. Cáliz de 5–6 mm, dientes triangulares de 1 mm, híspidos en el exterior; corola de 8–12 mm, exteriormente con pubescencia adpresa, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 5 mm, base del estilo no ensanchada. Fruto no visto.

Distribución geográfica. Chile en las regiones IV, V, VI, y VII y la Región Metropolitana, en la provincia fitogeográfica Chilena Central (Fig. 1D). Se ha coleccionado a 1900 m.

Fenología. Florece en verano.

Observaciones. Especie muy similar a *Junellia pseudojuncea* por su porte, diferenciándose principalmente por los entrenudos mayores y hojas apenas desarrolladas y brácteas lineares.

La variedad se ha sinonimizado porque se pudo observar la presencia de hojas enteras y algunas divididas en un mismo ejemplar de herbario.

Material adicional examinado. CHILE. **Región Metropolitana**: Cordillera de Santiago, Valle Largo de Las Condes, *Grandjot 34a* (SI). **IV Región**: Limari, Cordillera de Ovalle, Las Majadas, *Jiles 6406* (CONC). **V Región**: Los Andes, Juncal, *Castillo s.n.* (CONC). **VI Región**: Colchagua, Termas del Flaco, *Montero 9627* (CONC). **VII Región**: Curico, Valle superior Río Teno, *Zöllner 5183* (CONC).

26. Junellia pseudojuncea (Gay) Moldenke, Lilloa 8(2): 418. 1942. Basónimo: *Verbena pseudojuncea* Gay, Fl. Chile 5: 19. 1876. *Verbena spathulata* var. *pseudojuncea* (Gay) Reiche, Fl. Chile 5: 280. 1910. TIPO: [Chile.] Coquimbo: Arqueros, in collibus, dic. 1836, *C. Gay 1081*, SGO 54710 (lectotipo, designado por Acevedo de Vargas (1951: 60), SGO!). Figura 7A–D.

Thryothamnus junciformis Phil., Anales Univ. Chile 90: 618. 1895. *Verbena junciformis* (Phil.) Reiche, Fl. Chile 5: 281. 1910. TIPO: Chile. "Ad originum flumiis Torca, in dpto. Ovalle", 1889–1890, *G. Geisse s.n.*, SGO 42533 (lectotipo, designado por Acevedo de Vargas (1951: 60), SGO!).

Arbustos de 1–1.5 m de alt., tallos aparentemente áfilos, de sección circular, entrenudos hasta de 10 cm, generalmente sin desarrollo de braquiblastos. Hojas de 5–10 × 1–2 mm, reducidas, sésiles, lineares, enteras, a veces tripartidas, glabras. Monobotrios o dibotrios plurifloros, espigas contraídas, alargadas en la madurez hasta 3–4 cm, flores perfumadas; brácteas de 5 × 1 mm, lineares, agudas, pubescentes en el dorso, todas similares entre sí, iguales o apenas menores que la longitud del cáliz. Cáliz de 4–4.5 mm, cilíndrico, híspido, dientes de 1 mm, triangulares; corola de 8–11 mm, violáceo clara, escasos pelos en la parte superior externa del tubo y en los lóbulos, fauce glabra, pelos retrorsos en el interior del tubo; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 5.5–6 mm, base del estilo no ensanchada. Clusas de 5–5.5 mm, glabras, cara dorsal reticulada, caras comisurales papilosas.

Distribución geográfica. Crece en Chile en las Regiones III y IV, entre los 2000 y 2500 m, en campos pedregosos. Fitogeográficamente habita en las provincias del Desierto, distritos Coquimbano, de los Cardonales y Central (Fig. 1E).

Iconografía. Botta, 1989: 387, fig. 6.

Observaciones. Este taxón es muy similar a *Junellia spathulata*; se diferencia principalmente en las brácteas florales ovales. Se asemeja también a *J. lavandulaefolia*, ver observaciones en esa especie.

El ejemplar *C. Gay 1081* había sido nombrado por Acevedo de Vargas (1951: 60), como "Cotypus"; dado que la autora no hace referencia a otro ejemplar como tipo de la especie, se considera al mismo como lectotipo.

Material adicional examinado. CHILE. **III Región**: Huasco, Km 24 río del Estrecho, *Arancio 94183* (CONC). **IV Región**: Limari, planta eléctrica Los Molles, Bocatoma, *Ricardi 753* (CONC 27788); río Flamenca, *Jiles 2905* (SI).

27. Junellia selaginoides (Kunth ex Walp.) Moldenke.

27a. Junellia selaginoides (Kunth ex Walp.) Moldenke var. **selaginoides**, Prelim. Alph. List Inval. Incor. Sci. Names Verben. Avisen.: 48. 1940. Basónimo: *Verbena selaginoides* Kunth ex

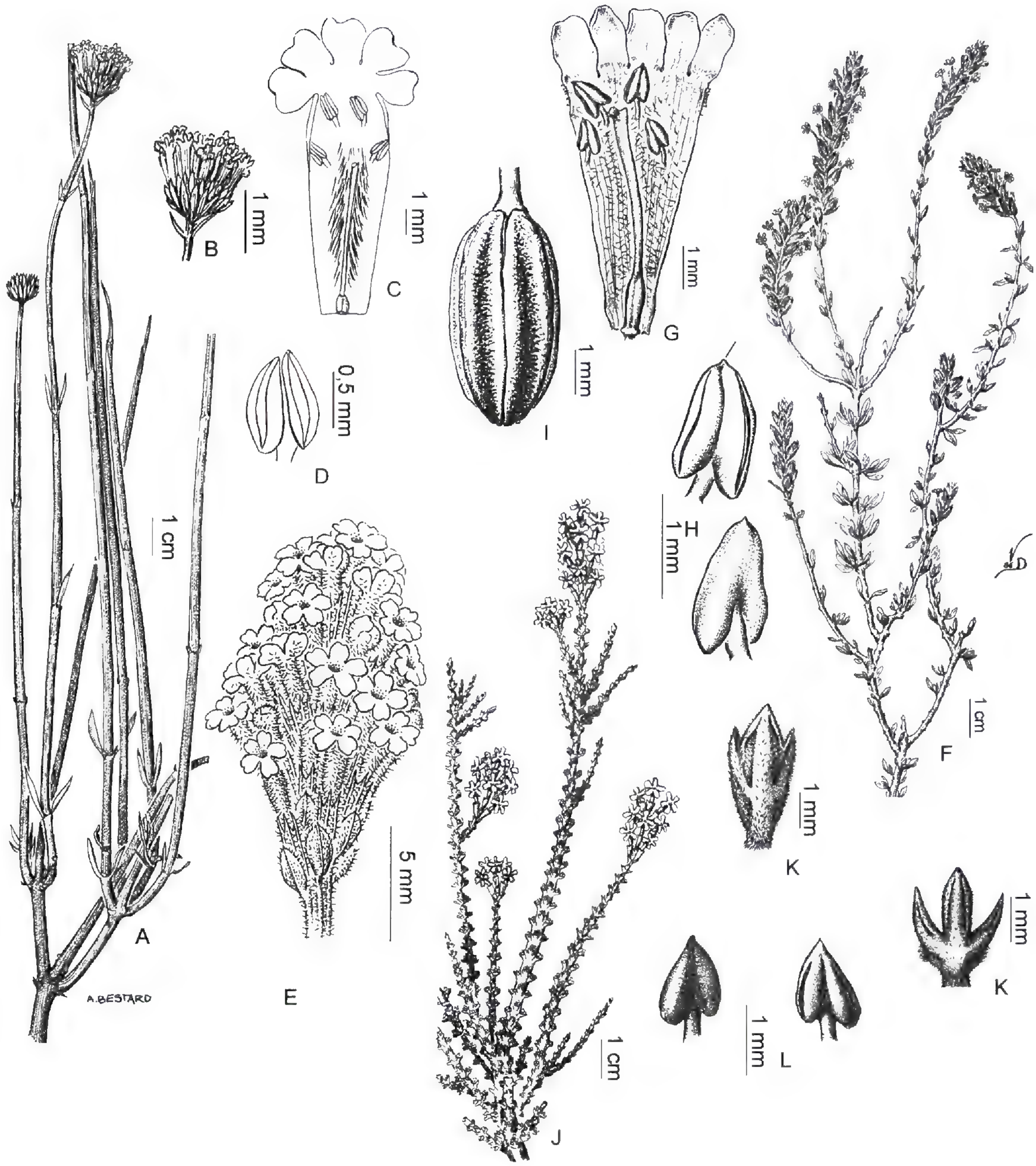


Figura 7. A–D. *Junellia pseudojuncea*. —A. Aspecto general. —B. Inflorescencia. —C. Corola abierta. —D. Antera. A–D de *Ricardi 753*, CONC 27788; D tomado de Botta, 1989: 387, fig. 6, con el permiso de F. Zuloaga. —E. *Junellia lavandulaefolia*. Inflorescencia (de *Grandjot 34a*). F–I. *Junellia aspera* var. *aspera*. —F. Aspecto general. —G. Corola abierta. —H. Anteras. —I. Fruto. F–I de *Boelcke 15673*. J–L. *Junellia tridens*. —J. Aspecto general. —K, K′. Hojas. —L. Anteras. J–L de *Soriano 3388*; L tomado de Botta, 1999: 191, fig. 141, con el permiso de F. Zuloaga.

Walp., Repert. Bot. Syst. 4: 15. 1845. TIPO: Chile. Coquimbo: 1839, *C. Gay s.n.* (lectotipo, aquí designado, G!, G foto FM 7856 SI!). Figura 8A–D.

Verbena polycephala Turcz., Bull. Soc. Imp. Naturalistes Moscou 36(2): 194. 1863. TIPO: Chile. Coquimbo: *T. Bridges 1353* (holotipo, K!, K foto SI!; isotipos, BM!, SI!).

Arbustos de 0.5–0.8 m de alt.; ramas erguidas, entrenudos de 0.5–2.5 cm, estriados, de sección poligonal, con pubescencia de pelos suaves, en general adpresos, retrorsos, con braquiblastos desarrollados. Hojas homomorfas, de 5–15(–17) × 1–1.5 mm, subopuestas, sésiles, lineares, subcarnosas, margen revoluto, cara adaxial con pubescencia escabrosa, cara abaxial con pubescencia híspido-glandulosa. Monobotrios plurifloros en espigas contraídas, alargadas a la madurez hasta de 1–3.5 cm; brácteas de 7–9 × 1.5–2.5 mm, ovado-angostas, con el margen apenas revoluto, ciliado, pubescencia

Figura 8. A–D. *Junellia selaginoides* var. *selaginoides*. —A. Aspecto general. —B. Flor. —C. Corola abierta. —D. Anteras. A–D de CONC 30923. E–H. *Junellia selaginoides* var. *illapelina*. —E. Aspecto general. —F. Flor. —G. Corola abierta. —H. Fruto. E–H de *Goodspeed 165120*.

semejante a la de las hojas, con pelos glandulares más abundantes. Flores perfumadas. Cáliz de 5–5.5 mm, con dientes subulados, en general mayores de 1 mm, híspido sobre los dientes y costillas, más adpresos en el resto de la superficie, con abundantes pelos glandulares; corola de 7.5–9.5 mm, pubescente exteriormente con escasos pelos glandulares, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 5.5–7 mm, base del estilo no ensanchada y más o menos hundida entre las clusas. Clusas de de 2.5–3 mm, ápice obtuso, cara dorsal reticulada, pardo-obscura, caras comisurales papilosas.

Distribución geográfica. Chile, en las Regiones II y IV, crece en la zona litoral entre los 25 S y 30 S y entre los 50 y 150(500) m; en la provincia fitogeográfica del Desierto, distrito Coquimbano (Fig. 1F).

Observaciones. Las restantes especies de la sección se diferencian por la falta de desarrollo de braquiblastos.

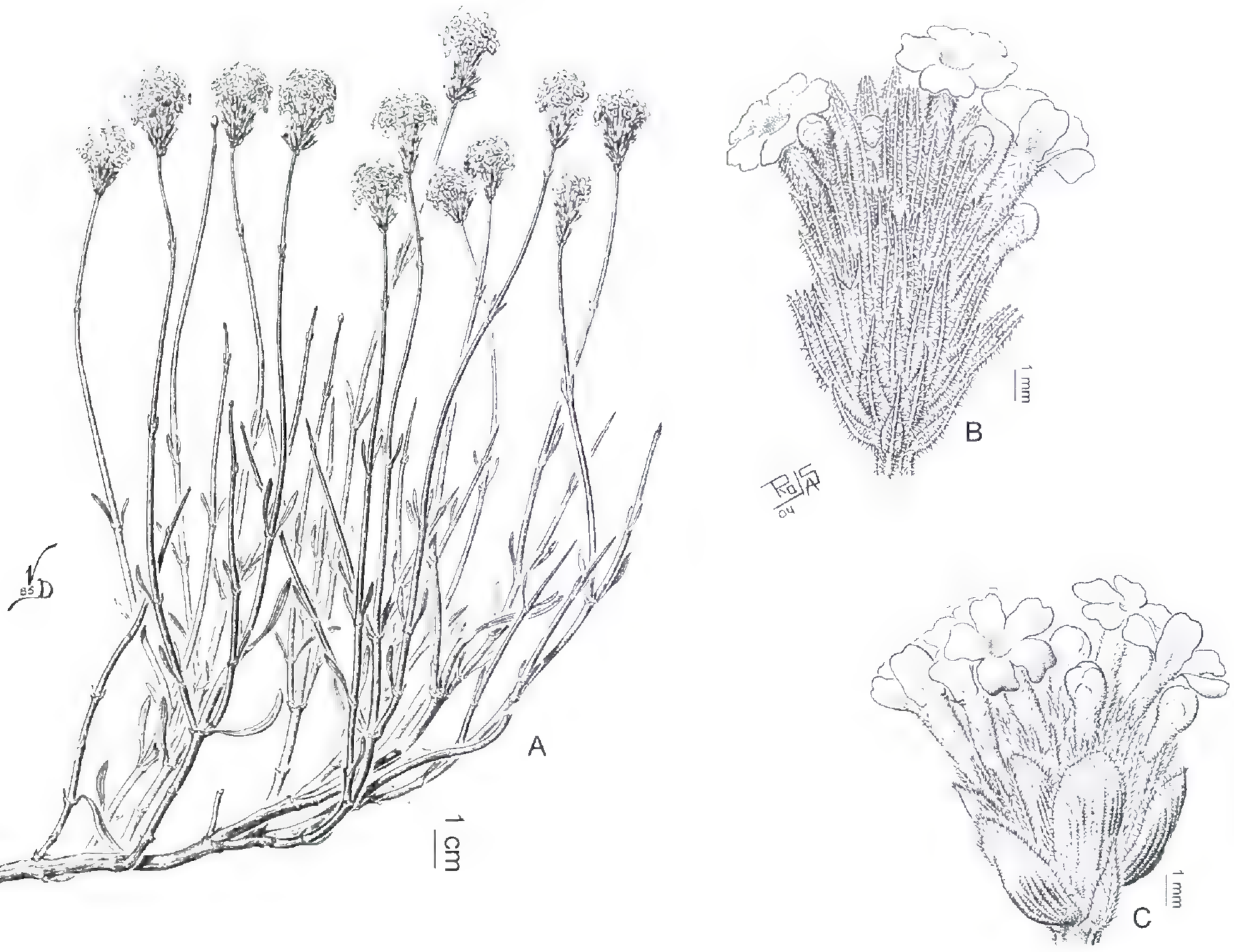


Figura 9. A, B. *Junellia spathulata* var. *spathulata*. —A. Aspecto general (de *Cabrera 33382*). —B. Detalle de la inflorescencia (de *Seijo 1713*). —C. *Junellia spathulata* var. *glauca*. Detalle de la inflorescencia (de *Ruíz Leal 26835*).

En la descripción original, Walpers describe la especie refiriendo el nombre a un binomio usado por Kunth en mss. Para la lectotipificación se eligió un ejemplar que lleva una etiqueta manuscrita por Kunth y que coincide con la descripción original.

En el ejemplar *Bridges 1353* depositado en K, figura la localidad Concepción, lo que probablemente sea un error en la etiqueta (Botta mss.).

Material adicional examinado. CHILE. **II Región**: Antofagasta, Cerro Azul, *Grandjot 4456 a* (SI). **IV Región**: Coquimbo, Hacienda Talinay, *Garaventa 4459* (SI).

27b. Junellia selaginoides var. **illapelina** (Phil.) Botta, Hickenia 2: 128. 1995. Basónimo: *Verbena illapelina* Phil., Anales Univ. Chile 90: 612. 1895. *Verbena selaginoides* var. *illapelina* (Phil.) Reiche, Fl. Chile 5: 288. 1910. *Junellia illapelina* (Phil.) Moldenke, Phytologia 2: 51. 1941. TIPO: Chile. Cordillera de Illapel, La Polcura, ene. 1888, s. col., SGO 54727 (lectotipo, designado por Botta (1989: 391), SGO!, SGO foto SI!; isotipo, P!). Figura 8E–H.

Difiere de la variedad típica por el tamaño de las hojas: de 1.5–5 mm en los macroblastos y de 1.5–2 mm en los braquiblastos; el cáliz con dientes triangulares y agudos de 0.6–1 mm y escasos pelos glandulares.

Distribución geográfica. Chile, en la Región III y IV, crece en cerros y quebradas entre los 600 y 1500 m; en la provincia fitogeográfica del Desierto, distrito Coquimbano (Fig. 1F).

Material adicional examinado. CHILE. **III Región**: Huayco O. Mineral, Los Cristales, Quebrada Las Salinas, *Marticorena 1723* (CONC). **IV Región**: Coquimbo, Linari, Ovalle, *Jiles 1919* (SI).

28. Junellia spathulata (Gillies & Hook. ex Hook.) Moldenke.

28a. Junellia spathulata (Gillies & Hook. ex Hook.) Moldenke var. **spathulata**, Lilloa 5: 401. 1940. Basónimo: *Verbena spathulata* Gillies & Hook. ex Hook., Bot. Misc. 1: 162. 1829. TIPO: Argentina. Mendoza: en valle de las Leñas Amarillas, in E ascent to El Planchon, s.f., *J. Gillies s.n.* (lectotipo, designado por Botta (1989: 392), K!, K foto SI!; isotipo, SI!). Figura 9A, B.

Verbena spathulata Gillies & Hook. ex Hook. var. *parviflora* Schauer in DC., Prodr. 11: 543. 1847. *Diostea stenophylla* Miers, Trans. Linn. Soc. London Bot. 27: 105. 1869. TIPO: Chile. Andes of Chile, *T. Bridge 1220* (lectotipo, aquí designado, K!, K foto SI!; isotipo, BM!).

Arbustos de 70 cm alt.; tallos erectos, glabros, sección circular, estriados, aparentemente áfilos, sin

desarrollo de braquiblastos. Hojas de 2–20 × 2–4 mm, enteras, lineares, sésiles, margen algo revoluto, carnosas, generalmente glabras, a veces estrigosas en ambas superficies. Monobotrios plurifloros en espigas contraídas densas, alargadas en la madurez hasta 4 cm, brácteas de 2–3 × 0.5–0.6 mm, angostamente ovadas, ápice agudo y rígido, siempre más cortas que el cáliz y todas similares, margen no membranáceo. Cáliz de 5–6.5 mm, glabro en la base, híspido hacia el ápice, con dientes desiguales, agudos, pubescentes exteriormente; corola lila, de 9 mm, exteriormente con pelos retrorsos, tubo recto, glabro, fauce glabra, limbo patente; par superior de estambres sin apéndices conectivales, conectivo no superando la longitud de las tecas; gineceo de 6.5 mm, base del estilo no ensanchada. Clusas de 4 × 1.2 mm, glabras, con alas laterales pequeñas, cara dorsal reticulada, caras comisurales papilosas.

Distribución geográfica. Crece en la región andina de Argentina, sur de Mendoza y Neuquén, y en Chile, en las regiones Metropolitana, VI, y VII. Habita entre los 1900 y 2200 m, en la provincia fitogeográfica Altoandina, distrito Altoandino-cuyano (Fig. 2B).

Fenología. Florece en verano especialmente en los meses de enero y febrero.

Iconografía. Botta, 1999: 183, fig. 136.

Observaciones. Tanto la variedad típica como la variedad *glauca*, se diferencian principalmente de *Junellia lavandulaefolia* y de *J. pseudojuncea*, por la morfología de las brácteas.

El análisis de ejemplares de *Verbena spathulata* var. *parvifolia*, en el herbario K, fue realizado por Botta, quien en sus manuscritos dejó indicado el citado ejemplar como lectotipo, ya que las características del mismo se corresponden con la descripción original. Por otra parte el mismo ejemplar fue citado como tipo de *Diostea stenophyla* en la descripción original.

Material adicional examinado. ARGENTINA. **Mendoza**: Dpto. Malargüe, Río Grande, 60 km al N de Las Loicas, *Cabrera 33492* (SI). **Neuquén**: Dpto. Ñorquinco, Puerta de Trolope, *Sanso 121* (SI). CHILE. **Región Metropolitana**: Santiago, Refugio Lo Valdes, Valle de Maipú, *Garaventa 6286* (CONC). **VI Región**: Vegas del Flaco, camino a la mina, *Ricardi 3246* (SI). **VII Región**: Curicó, Cord. Volcán Peteroa, *Werderman 577* (BM, SI).

28b. Junellia spathulata var. **glauca** (Gillies & Hook. ex Hook.) Botta, Hickenia 2: 128. 1995. Basónimo: *Verbena glauca* Gillies & Hook. ex Hook., Bot. Misc. 1: 163. 1829. *Junellia glauca* (Gillies & Hook. ex Hook.) Moldenke, Lilloa 5: 396. 1940. TIPO: Argentina. Mendoza: near the River Diamante, *J. Gillies s.n.* (holotipo, K!, K foto SI!). Figura 9C.

Verbena spathulata var. *grandiflora* Schauer en DC., Prodr. 11: 543. 1847, syn. nov. *Verbena ochreata* Briq., Annuaire Conserv. Jard. Bot. Genève 4: 8. 1900. *Junellia spathulata* var. *grandiflora* (Schauer) Botta, Hickenia 2: 128. 1995. TIPO: Chile. *Gay s.n.* (lectotipo, aquí designado, P!; isotipo, SI!).

Diostea filifolia Miers, Trans. Linn. Soc. London Bot. 27: 107. 1869. TIPO: Chile. Cordillera de Santiago, *Germain s.n.* (lectotipo, aquí designado, K!; isotipos, BM!, P!).

Verbena glauca Gillies & Hook. ex Hook. var. *cisandina* Niederl. en Lorentz & Niederl., Exp. Río Negro Bot.: 265. 1881. *Junellia glauca* (Gillies & Hook. ex Hook.) Moldenke var. *cisandina* (Niederl.) Moldenke, Lilloa 5: 396. 1940. TIPO: Argentina. Neuquén: "frecuente en el valle del río Neuquén y en la sierra cerca de la embocadura del río Curruleufu", 23 jun. 1879, *Niederlein s.n.* (holotipo, CORD!).

Se diferencia de la variedad típica por las brácteas basales de 3–6 × 3–4 mm, ovadas u ovado-oblongas, margen membranáceo o no, ciliado, ápice agudo a punzante; brácteas superiores de 4–6 × 2–3.5 mm, mayores que la mitad de la longitud del cáliz, ovadas o angostamente ovadas, híspidas hacia la base, ápice mucronado, rígido u obtuso margen ciliado. Cáliz de 4.5–6 mm.

Cariología. $2n = 20$ (sub *Junellia glauca*, Covas & Schnack, 1947: 225, 229, fig. 30). $n = 10$ (Poggio et al., 1988: fig. 1A). $2n = 20$ (Botta & Brandham, 1993: 146, fig. 1A).

Distribución geográfica. Precordillera del S y W de Mendoza y Neuquén, centro de Chile, regiones Metropolitana, IV, V y VII; en las provincias fitogeográficas del Monte y Altoandina (Fig. 2B).

Fenología. Florece entre noviembre y enero.

Iconografía. Lorentz y Niederlein, 1881: tab. XII, II, fig. 6 (sub *Verbena glauca* var. *cisandina*).

Observaciones. La variedad *Verbena spathulata* var. *grandiflora*, fue lectotipificada en base a las observaciones realizadas por Botta, quien en sus manuscritos dejó indicado el citado ejemplar como lectotipo y se pudieron corroborar sus observaciones. En el caso de *Diostea filifolia*, el material citado en la descripción original, fue también analizado por Botta, quien en sus manuscritos dejó asentado la elección como lectotipo y que dicho material se corresponde a *Junellia spathulata* var. *grandiflora*.

Material adicional examinado. ARGENTINA. **Mendoza**: Dpto. Malargüe, Los Ranquiles, *Martínez Crovetto 6226* (SI). **Neuquén**: Dpto. Catán Lil, cerca de 12 km de Las Cortaderas hacia Charahuilla, *Correa 7913* (SI). CHILE. **Región Metropolitana**: Santiago, Los Condes, *Gillies s.n.*,

LP 8470, 8469 (LP). **IV Región**: Coquimbo, Illapel, quebrada la Vega escondida, *Goodspeed 16594* (SI). **V Región**: Los Andes, Los Maitenes, Río Colorado, *Zöllner 6528* (CONC). **VII Región**: Curicó, Laguna del Planchón, *Zöllner 5991* (CONC).

Junellia subgén. **Thryothamnus** secc. **Dentium** P. Peralta & Múlgura, sect. nov. *Junellia* subgén. *Thryothamnus* secc. *Junelliopsis* Botta, syn. nov. Darwiniana 29: 392. 1989, p.p., excluyendo el tipo. TIPO: *Verbena tridens* Lag. [= *Junellia tridens* (Lag.) Moldenke].

Arbustos altos, ramas de sección poligonal o subcircular. Hojas dimorfas: las de los macroblastos, enteras o tripartidas, espiniformes; las de los braquiblastos con lámina desarrollada, enteras, carnosas, no espiniformes. Monobotrios en espigas cilíndricas, alargadas o contraídas. Corola con fauce densamente pubescente, pelos diferentes a los del interior del tubo corolino; estambres con conectivo generalmente de mayor longitud que las tecas y sin apéndices conectivales.

Observaciones. Las especies de esta sección incluyen todas las especies de *Junellia* subgén. *Thrythamnus* secc. *Junelliopsis* sensu Botta, excluido el tipo, *J. bryoides* que se transfirió al subgénero *Junellia*. *Junellia tridens* se designa como nueva especie tipo por presentar los caracteres típicos de la sección.

29. Junellia asparagoides (Gillies & Hook. ex Hook.) Moldenke, Lilloa 5: 395. 1940. Basónimo: *Verbena asparagoides* Gillies & Hook. ex Hook., Bot. Misc. 1: 165. 1829. TIPO: Argentina. Mendoza: "near the upper...from Portezuelo to the valley of Uspallata", s.f., *Gillies s.n.* (lectotipo, aquí designado, K!, K foto SI!).

Verbena diversifolia Kuntze, Revis. Gen. Pl. 3(2): 255. 1898. *Junellia diversifolia* (Kuntze) Moldenke, Lilloa 5: 395. 1940. TIPO: Argentina. Mendoza: Paso Cruz, 2000 m, s.f., *Kuntze 5* (holotipo, NY!).

Arbustos de 1–1.5 m de alto, con ramas erguidas, flexibles, pubescentes hacia el ápice; entrenudos de 0.5–1 cm. Hojas dimorfas, las de los macroblastos de (2–)3–4(–8) mm, tripartidas, espiniformes, lóbulo central de mayor longitud que los laterales; hojas de los braquiblastos 9(–13) × 1–1.5(–3) mm, enteras, subcrasas, obovadas a linear-oblongas, ápice obtuso a veces subobtuso, con un mucrón breve y base cuneada, superficie escabrosa. Monobotrios en espigas plurifloras, de 3 cm; brácteas de 2.5–8(–9) mm, ovadas o lineares, subconnadas, ápice agudo a punzante, a veces acuminadas, margen ciliado. Cáliz tubuloso, 5-dentado, con tubo de 3–4.5 mm, marcadamente 5-costado; dientes de 0.5–3 mm, subiguales, subulados, rígidos y algo recurvos en el ápice, pubescencia de pelos cortos, esparcidos, blanquecinos, especialmente entre las costillas; corola lila, de 8.5–12.5 mm, con pelos suaves y cortos, más o menos erectos, adpresos, desde la mitad superior hasta la base de los lóbulos; fauce con pelos claviformes; par superior de estambres sin apéndices conectivales, conectivo de igual longitud que las tecas; gineceo de 5–6.6 mm, base del estilo no ensanchada, ápice glabro. Clusas de 2.8–3 mm, ápice obtuso, angostándose hacia la base, cara dorsal convexa, castaño oscura, estriada, caras comisurales papilosas, blanquecinas.

Cariología. $2n = 20$ (*Junellia asparagoides*, Covas & Schnack, 1946: 156, 159, fig. 2G).

Nombre vulgar. "Barba de viejo" (Ruíz Leal, 1972: 38).

Distribución geográfica. Habita en Argentina en las provincias de Catamarca, Jujuy, La Rioja, Mendoza, Salta, San Juan y Tucumán y en Chile en las regiones III y IV. Crece entre los 2000 y 2800 m en la provincia Prepuneña y asciende por las quebradas llegando hasta la Puna (Fig. 1A).

Fenología. Florece en verano desde diciembre hasta febrero.

Iconografía. Sanzin, 1919: 111, fig. 15 (flor sub *Verbena asparagoides*); Ruíz Leal, 1972: 37, lám. 9, fig. 27 (sub *Verbena asparagoides*); Botta, 1984: 337, fig. 2 (sub *Verbena asparagoides*); Botta, 1993: 70. fig. 30.

Observaciones. La especie se caracteriza por las hojas de los macroblastos espiniformes tripartidas y los dientes del cáliz subulados y rígidos. En *Junellia cedroides*, los dientes son triangulares, aunque ambas especies presentan hojas dimorfas.

Los ejemplares de Gillies, depositados en K, fueron estudiados por Botta, quien eligió el ejemplar citado como lectotipo, porque tiene los caracteres propios de la especie y la etiqueta se corresponde en parte con la descripción original: "Hab. In summum fere jugum Uspallata apud Cerro del Portezuelo: alt. 10,000 ped".

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Belén, Cuesta de Randolfo, *Cabrera 32449* (SI). **Jujuy**: Dpto. Tilcara, Quebrada de Huasamayo, *Cabrera 31563* (SI). **La Rioja**: Dpto. Famatina, Sierra de Famatina, entre Los Corrales y Los Berros, a 3.5 km de la primera, *Biurrun 7091* (SI). **Mendoza**: Dpto. Las Heras, paramillo de Uspallata, *J. H. Hunziker 3071* (SI). **Salta**: Dpto. Cachi, desde ruta 40, camino interno hacia Las Paylas, *Cialdella 284* (SI). **San Juan**: Dpto. Angaco, Sierra de Pie de Palo, camino a Mogote de los Corralitos, *Kiesling 3108* (SI). **Tucumán**: Dpto. Tafí, La Cienaguita, Cumbres Calchaquíes, *Rodríguez 1218* (SI). CHILE. **III Región**: Copiapó, Quebrada El Patón, *Arancio 657* (CONC). **IV Región**: Ovalle, Cordillera Ovalle, *Jiles 2898* (SI); Coquimbo, Baños del Toro, Estero de Huanca, *Werdermann 236* (SI).

30. Junellia cedroides (Sandwith) Moldenke, Lilloa 5: 394. 1940. *Verbena cedroides* Sandwith, Kew Bull. 1927: 184. 1927. TIPO: Argentina. Neuquén, Zapala: Zapala, 27 nov. 1925, *H. F. Comber 189* (holotipo, K!, K foto SI!; isotipos, BM no visto, LP!, TEX no visto).

Arbustos espinosos de 0.15–1 m de alt.; ramas jóvenes casi glabras. Hojas dimorfas, las de los macroblastos de 4–5 × 1.8–2.2 mm, 3-partidas, lóbulos angostamente elípticos, ápice agudo, punzante, connadas entre sí en las ramas jóvenes; hojas de los braquiblastos de 2.5–3 × 0.5–0.7 mm, espatuladas, subcarnosas, ápice obtuso y base decurrente, casi glabras. Monobotrios en espigas plurifloras; brácteas de 3–4 mm, ovadas, ápice obtuso, base decurrente, ciliadas en el margen. Cáliz de 6–6.5 mm, campanulado, dientes triangulares, ápice agudo, punzante, pubescencia de pelos cortos, antrorsos, ralos; corola de 8–11 mm, lila, externamente con pubescencia blanca, pelos muy cortos y suaves, adpresos, antrorsos, densos, menos en la base del tubo y ápice de los lóbulos, internamente pubescente, fauce con pelos claviformes; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 7.5–8 mm, obovoide, ápice glabro, base del estilo no ensanchada. Clusas de 4–4.2 mm, glabras, ápice obtuso y base angostada, cara dorsal estriada casi negra, caras comisurales papilosas, blancas.

Distribución geográfica. Crece en las provincias de Neuquén y Río Negro, en provincia fitogeográfica Patagónica, alrededor de los 1200 m (Fig. 1B).

Fenología. Florece en diciembre.

Iconografía. Botta, 1999: 167, figs. 122, 169.

Observaciones. Especie que se caracteriza por tener los dientes del cáliz triangulares y de ápice agudo y punzante, que la diferencia de *Junellia asparagoides*. Es afín también a *J. tridens*, de la cual se diferencia por tener el ovario glabro.

Material adicional examinado. ARGENTINA. **Neuquén**: Dpto. Zapala, de Zapala a Primeros Pinos, *Cabrera 32850* (SI). **Río Negro**: Dpto. Pilcaniyeu, Laguna Blanca, ruta 40, 28 dic. 1963, *Schajovskoy s.n.*, SI 25664 (SI).

31. Junellia hystrix (Phil.) Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. Basónimo: *Verbena hystrix* Phil., Anales Univ. Chile 90: 610. 1895. TIPO: Chile. Atacama: Acerillos, desierto de Atacama, nov. 1844, *Villanueva s.n.*, SGO 42514 (lectotipo, designado por Acevedo de Vargas (1951: 55), SGO!, SGO foto SI!). Figura 10A–D.

Arbusto de 0.50–1 m alt.; tallos jóvenes densamente vellosos, con pelos adpresos, glabrescente a la madurez. Hojas dimorfas, trisectas, las de los macroblastos opuestas con lóbulos de 3–3.5 × 1 mm, ovados, ápice atenuado, punzante, nervio medio prominente en la cara abaxial, pelos glandulares en ambas caras, cara adaxial villosa en la base, margen ciliado; hojas de los braquiblastos imbricadas, carnosas, con lóbulos de 1.5 × 0.5 mm, ovado-angostos, ápice agudo, rígido, nervio medio no prominente, pubescencia similar a las hojas de los macroblastos. Monobotrios en espigas plurifloras, de 1–1.5 cm, acrescentes en la fructificación hasta 4–6 cm; brácteas de 3.5–8 × 1–1.5 mm, enteras, ovadas, ápice generalmente punzante. Cáliz de 7–8 mm, con costillas prominentes, ciliado en el margen superior, superficie con pelos glandulares y no glandulares, patentes, dientes subulados, pungentes hasta 3 mm; corola de 11–12 mm, con pelos desiguales y de densidad variable en la cara externa del tubo, fauce con pelos claviformes; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 8 mm, ápice glabro, base del estilo no ensanchada, levemente hundida entre las clusas. Clusas de 3–3.5 mm, glabras, ápice obtuso, cara dorsal negruzca, reticulada, caras comisurales blancas y papilosas.

Nombre vulgar. "Yote" (Acevedo de Vargas, 1951: 55).

Distribución geográfica. Crece en el norte de Chile, en la Región II y III y en San Juan (Argentina); en el dominio Andino-patagónico, provincia fitogeográfica del Desierto, hasta los 3500 m (Fig. 1D).

Fenología. Se ha coleccionado en flor en los meses del verano.

Observaciones. Junellia hystrix es afín a *J. asparagoides*, por las hojas de los macroblastos rígidas y punzantes y los rígidos dientes del cáliz; *J. asparagoides* presenta un porte mayor y hojas enteras en los braquiblastos.

Asimismo esta especie fue considerada sinónimo de *Junellia bryoides* (ver observación en este taxón).

Material adicional examinado. ARGENTINA. **San Juan**: Dpto. Calingasta, Los Morrillos (Reserva Privada Fundación Vida Silvestre), *Fortunato 5602* (SI). CHILE. **II Región**: Antofagasta, Cordillera Domeyko, Salar Punta Negra, *Chong s.n.*, Herbario Gunkel 49305 (CONC). **III Región**: Atacama, Chañaral: camino de potrerillos a Pedernales, Km 14, *Ricardi 1589* (LP); Cuesta de Pedernales, *Behn s.n.*, CONC 23739 (CONC, SI).

32. Junellia tetragonocalyx (Tronc.) Moldenke, Phytologia 3: 167. 1949. Basónimo: *Verbena tetragonocalyx* Tronc., Darwiniana 8: 481. 1949. TIPO: Argentina. Chubut, Paso de Indios: 23 km al E de El Sombrero, 6 ene. 1948, *Soriano 2839* (holotipo, SI!; isotipo, K no visto, K foto SI!).

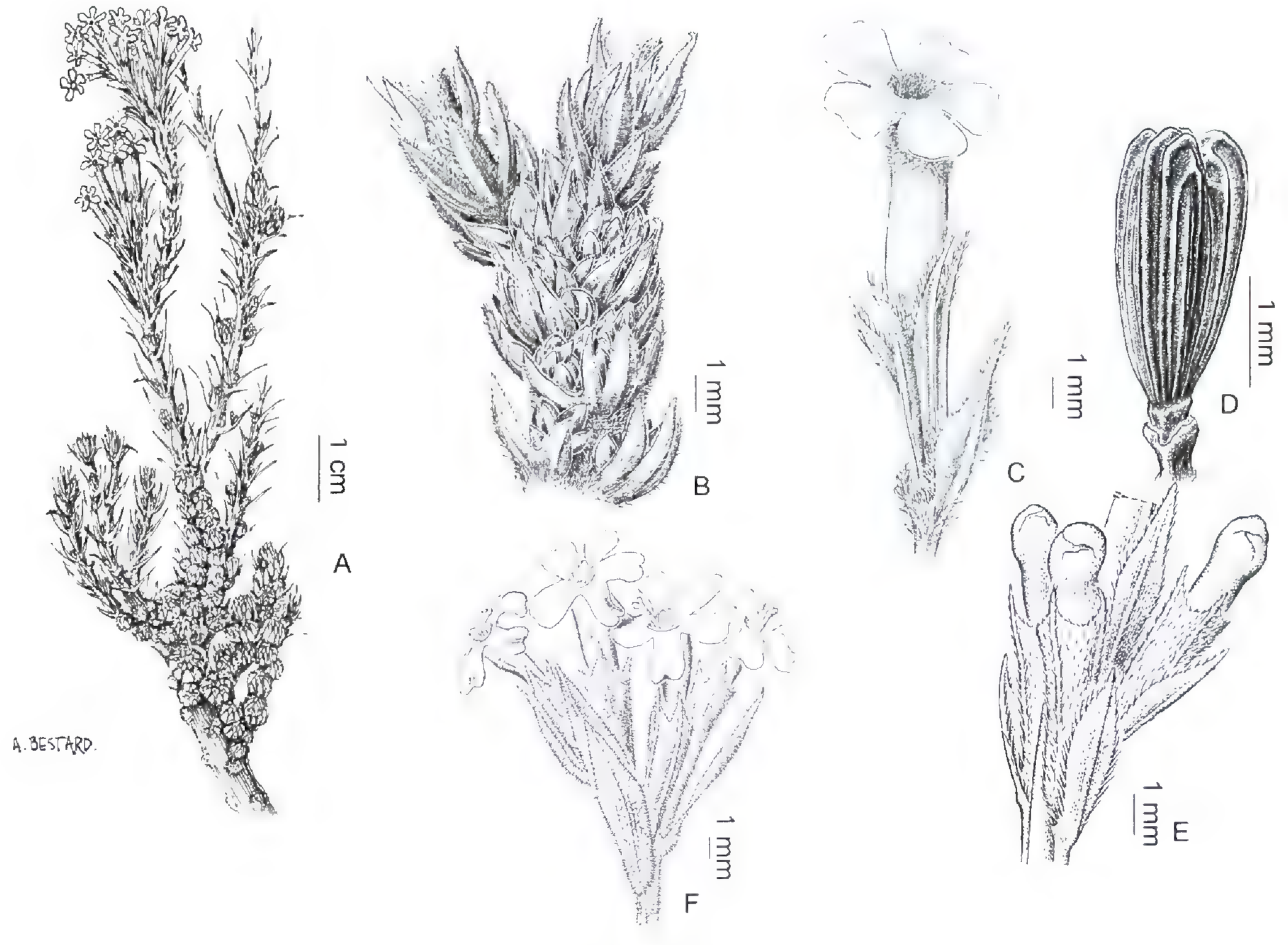


Figura 10. A–D. *Junellia hystrix*. —A. Aspecto general (de *Zöllner 4397*). —B. Detalle de una rama. —C. Flor y bráctea. B, C de *Behn 23739*. —D. Fruto (de CONC 31655). —E. *Junellia ligustrina* var. *ligustrina*. Inflorescencia (de *M. H. Nat. San Rafael 3615*). —F. *Junellia ligustrina* var. *lorentzii*. Inflorescencia (de *Daciuk 25*).

Arbusto de 0.8–1.2 m alt., oscuro, subglabro; macroblastos con entrenudos de 4–10 mm, braquiblastos de 3–4 mm. Hojas dimorfas, las de los macroblastos de 2–2.5 × 1–1.5 mm, enteras, sésiles, connadas, agudas, en general punzantes al envejecer, cara abaxial con nervio medio prominente, recurvas; hojas de los braquiblastos enteras de 1.5 × 0.5–1 mm, carnosas, sésiles, angostamente oblongas, obtusas, a veces brevemente apiculadas. Monobotrios en espigas pluriflloras; brácteas de 2.5–5 × 2–3.5 mm, anchamente ovadas, obtusas y apiculadas, margen ciliado. Cáliz de 6–8 mm, con cuatro costillas, dientes subulados recurvos, ensanchado hacia el ápice, pubescente en la mitad superior; corola de 11–12 mm, blanco-amarillenta, pubescente en la mitad superior, fauce con pelos moniliformes; estambres con conectivo de mayor longitud que las tecas; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 9–10 mm, ápice glabro, base del estilo no ensanchada, hundida entre las clusas. Clusas de 4.5–5 mm, cilindricas, ápice obtuso, cara dorsal oscura, caras comisurales blancas, papilosas.

Distribución geográfica. Se la encuentra en Río Negro y Chubut; en la provincia fitogeográfica Patagónica (Fig. 2C).

Fenología. Florece y fructifica en verano.

Iconografía. Troncoso, 1949: 481, 482, figs. 1, 2 (sub *Verbena tetragonocalyx*); Botta, 1999: 182, 191, fig. 142.

Observaciones. Especie muy similar a *Junellia tridens* de la que se diferencia por tener hojas partidas en los macroblastos, el cáliz 5-dentado, 5-costado, clusas pubescentes en el ápice.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Mártires, Las Plumás, almacén El Guanaco, *Soriano 3418* (SI). **Río Negro**: Dpto. San Antonio, cerca de 2 km de Gendarmería Nacional en Sierra Grande, camino a Bocamina, *Crespo 2471* (BAB).

33. Junellia tridens (Lag.) Moldenke, Lilloa 5: 402. 1940. Basónimo: *Verbena tridens* Lag., Gen. Sp. Pl. 19. 1816. TIPO: Argentina. Chubut, Florentino Ameghino: a 7 km del cruce de Ruta Provincial 28 con Ruta Nacional 3, 24 feb. 1990, *N. Correa 10227* (neotipo, aquí designado, SI!). Figura 7J–L.

Verbena carroo Speg., Anales Soc. Ci. Argent. 15: 112. 1883. TIPO: Argentina. Santa Cruz: 17 ene. 1882, *C. Spegazzini 1125* (holotipo, LP!; isotipos, K!, SI!, K foto SI!).

Arbustos de 1–1.5 cm alt., negruzcos, erectos. Hojas dimorfas, las de los macroblastos de 4–4.5 × 3–3.5 mm, sésiles, trilobadas a tripartidas, lóbulos agudos, punzantes con margen revoluto; cara adaxial con pelos adpresos, antrorsos, la abaxial hispídula; hojas de los braquiblastos ca. 1.5 × 1.3 mm, sésiles, ovadas, enteras, carnosas, dísticas, agudas, con margen revoluto, cara adaxial lisa, hispídula, abaxial surcada longitudinalmente en la parte media, con pelos breves, adpresos. Monobotrios de 15–20 mm, en espigas plurifloras, sobre braquiblastos; brácteas de 2–3 mm, trilobadas, semejante a las hojas de los macroblastos. Cáliz de 5–5.5 mm, densamente hispídulo, dientes agudos, rígidos, los adaxiales muy breves, los abaxiales más desarrollados, de 0.5–1 mm; corola de 9.5–10 mm, lila a blanquecina, con pubescencia adpresa-retrorsa en el exterior de la mitad del tubo y de los lóbulos, fauce con pelos claviformes; lóbulos obtusos, emarginados; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 6–6.5 mm, base del estilo no ensanchada, hundida entre las clusas, ápice del ovario pubescente. Clusas de 4–4.5 mm, ápice obtuso, pubescente, cara dorsal reticulada, casi negra, caras comisurales blancas, papilosas.

Cariología. $2n = 20$ (sub *Verbena tridens*, Dollenz, 1976: 165, fig. 1, 166).

Nombre vulgar. "Mata negra" (de la Peña & Pensiero, 2004: 367).

Distribución geográfica. Crece en las provincias de Chubut, Río Negro y Santa Cruz y en Chile en la Región XII; en la provincia fitogeográfica Patagónica, en el distrito occidental (Fig. 2F). Suele ser elemento dominante de la estepa, se la encuentra en terrazas, mesetas y en el fondo de cañadones o valles fluviales cerca de mallines, forma matas hemisféricas.

Fenología. Florece entre diciembre y febrero.

Iconografía. Macloskie, 1905: pl. 23 (sub *Verbena carroo* Speg.); Soriano, 1956b: 368, fig. 8C; Botta, 1999: 191, fig. 141.

Observaciones. Especie muy afín a *Junellia cedroides* de la que se diferencia fundamentalmente por la pilosidad del ápice del gineceo.

Fue necesario designar un neotipo, porque el material que estudió Lagasca fue destruido. Se eligió el material de Correa, por ser uno de los más completos.

Material adicional examinado. ARGENTINA. **Chubut**: Dpto. Ameghino, Ruta Nac. 3, 10 km al S de Garayalde, *Botta 454* (SI). **Río Negro**: Dpto. Avellaneda, 18 km de Choele Choel hacia Río Colorado, *Correa 10519* (BAB). **Santa Cruz**: Dpto. Corpen Aike, Ruta 3, 32 km al S del Río Chico, *Botta 488* (SI); Dpto. Lago Argentino, *Soriano 3388* (SI). CHILE. **XII Región**: Magallanes, Kimiri Aike, *Pedersen 14430* (SI).

Junellia subgén. **Thryothamnus** secc. **Verticiflora** (Schauer) Botta, Darwiniana 29: 392. 1989. *Verbena* secc. *Verbenaca* ser. *Verticiflorae* Schauer in DC., Prodr. 11: 544. 1847. *Verbena* secc. *Junellia* ser. *Verticiflorae* (Schauer) Tronc., Darwiniana 18: 313. 1974. TIPO: *Verbena aspera* Gillies & Hook. ex Hook. [= *Junellia aspera* (Gillies & Hook. ex Hook.) Moldenke var. *aspera*].

Arbustos con ramas generalmente de sección poligonal o menos comúnmente tetragonal. Hojas con láminas variables, no espiniformes. Monobotrios o dibotrios en espigas, alargadas o contraídas. Corola con fauce glabra o con pelos semejantes a los del interior del tubo corolino; estambres con conectivo de mayor longitud que las tecas, con o sin apéndices conectivales desarrollados.

34. Junellia arequipensis (Botta) Botta, Darwiniana 29: 392. 1989. Basónimo: *Verbena arequipensis* Botta, Darwiniana 28: 237. 1987 [1988]. TIPO: Perú. Arequipa: encima de baños de Jesús, 23 abr. 1961, 2600–2700 m, *A. Ferreyra 14261* (holotipo, SI!).

Arbustos ramosos, de 1.20–1.80 m de alto; ramas jóvenes de sección poligonal, hispídulas. Hojas dimorfas, las de los macroblastos de 0.8–2 × 0.3–0.7 cm, opuestas, a veces alternas, enteras, coriáceas, subsésiles, elípticas, ápice agudo y base decurrente; cara adaxial estrigosa y abaxial híspida, especialmente sobre el nervio medio prominente; hojas de los braquiblastos iguales a las de los macroblastos pero menores, ocasionalmente, con lámina obovada, ápice obtuso. Monobotrios plurifloros, en espigas alargadas, de 10–14(–16) cm, cilíndricas; brácteas de 2.5–4 × 0.5 mm, angostamente ovadas a lineares, pubescentes, ciliadas en el margen. Cáliz de 4–5 mm, 5-dentado, dientes triangulares, desiguales de 0.4–0.5 mm, híspido sobre las costillas y estrigoso entre las mismas; corola de 13–15 mm, pardo rojiza y lóbulos amarillos, pubescente en la parte superior del tubo y base de los lóbulos, el resto de la superficie glabra; interior del tubo pubescente a la altura de la inserción de los estambres; fauce glabra; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 11–11.5 mm, base del estilo no ensanchada hundido entre las clusas. Clusas de 2.5–3 mm, pericarpo con alas a penas desarrolladas, cara dorsal reticulado-estriada, caras comisurales papilosas, blancas.

Nombre vulgar. "Romerillo", "capo colorado" (de *Arenas 84* [SI] y *Hinckley 54* [SI]).

Distribución geográfica. Coleccionada en Perú y Chile, entre los 15°S y 20°S, en laderas rocosas entre los 2200 y 4300 m (Fig. 1A). La región corresponde al dominio Andino-patagónico, provincias Altoandina y del Desierto, distrito de los Cardonales.

Fenología. Florece desde octubre hasta abril.

Iconografía. Botta, 1987: 238, fig. 1 (sub *Verbena arequipensis*).

Observaciones. Junellia arequipensis se caracteriza por sus flores, con tubo recurvo, de color pardo rojizo y muy perfumadas. Es afín a *J. aspera* diferenciandose por los dientes del cáliz más largos y los frutos marcadamente alados.

Material adicional examinado. CHILE. **I Región**: Arica, Zapahuira, *Escobar X–140* (SI); Tarapacá, Cord. Co. Japu, *Werdermann 1111* (SI). PERÚ. **Arequipa**: Arequipa, Estanquillo Ahogado, Chachani, *Arenas 84* (SI). **Cajatambo**: Lima, cerca de Churin, *Ferreyra 18706* (SI, USM). **Tacna**: Tarata, Tisaco, *Cano 8312* (USM).

35. Junellia aspera (Gillies & Hook. ex Hook.) Moldenke.

35a. Junellia aspera (Gillies & Hook. ex Hook.) Moldenke var. **aspera**, Lilloa 5: 393. 1940. Basónimo: *Verbena aspera* Gillies & Hook. ex Hook., Bot. Misc. 1: 163. 1829. TIPO: Argentina. Mendoza: Paramillo de Uspallata "near Los Hornillos, eastern descent from Paramillo de Uspallata", nov. 1822, *J. Gillies s.n.* (lectotipo, designado por Botta (1989: 392), K!; isotipo, BM!, BM foto SI!). Figura 7F–I.

Verbena ourostachya Briq., Annuaire Conserv. Jard. Bot. Genève 4(2): 20. 1900. TIPO: Argentina. Mendoza, San Rafael: Plantes des environs de St. Raphaël & de la Vallée du Rio Mendoza, jan. & fév. 1897, *E. Wilczek 51* (lectotipo, designado aquí, G!, G foto FM 24695 SI!; isotipos, F!, NY!).

Junellia tripartita Moldenke, Phytologia 3: 36. 1948, syn. nov. TIPO: Argentina. Mendoza, Santa Rosa: Las Catitas, 4 abr. 1942, *A. Ruíz Leal 7966* (holotipo, NY no visto; isotipos, BA!, MERL!).

Junellia longidentata var. *glandulosa* Botta, Hickenia 2: 128. 1995, syn. nov. TIPO: Argentina. La Rioja, Gral. Sarmiento: entre Jagüe y Sierra Punta Negra, 1800–2200 m, 23 ene. 1949, *A. Krapovickas 5480* (holotipo, SI!; isotipo, BAB!).

Arbustos ramosos de 0.80–1.20 m de alto. Ramas jóvenes de sección circular, divaricadas, glanduloso-pubescentes, al envejecer glabrescentes y espinescentes; corteza pardo-oscura, con estrías longitudinales. Hojas dimorfas, sésiles, las de los macroblastos a veces alternas, raramente verticiladas, generalmente lámina trisecta, lóbulo medio de 4.5–15 × 2–3.5(–7) mm, notablemente mayor que los laterales, elípticos a obovados, ápice agudo a obtuso, con la cara adaxial más densamente escabroso-glandulosa que la abaxial, ésta con el nervio medio prominente; hojas de los braquiblastos de (6–)8–14 × 1.2–1.3(–2) mm, enteras, elípticas a obovadas, con igual pubescencia que la de los macroblastos. Monobotrios plurifloros en espigas contraídas, de más de 5–10 cm a la madurez; brácteas de 3–5 mm, enteras, angostamente elípticas a lineares, ápice subulado, margen ciliado. Cáliz de 6–10 mm, híspido-glanduloso o escabroso-glanduloso, dientes desiguales, de 1–1.8 mm, subulados, el adaxial muy breve; corola de 6.5–10.5 mm, blanca, glabra en el exterior y escasamente glandulosa-pubescente en el interior, a la altura de la inserción de los estambres, fauce glabra; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 8 mm, base del estilo no ensanchada. Clusas de 5–7 × 2.5–3 mm, elipsoidales, glabras, con el pericarpio prolongado lateralmente en forma de dos alas membranáceas, conspicuas, cara dorsal pardo-oscura, lisa, caras comisurales blancas, papilosas.

Cariología. $2n = 60$ (*Junellia aspera*, Covas & Schnack, 1946: 156, 159, fig. 2H).

Nombre vulgar. "Capo colorado" (Moldenke & Moldenke, 1949: 7).

Distribución geográfica. Argentina, desde Catamarca hasta Río Negro (Fig. 1A). Habita en el dominio Chaqueño, provincia del Monte, en zonas áridas entre los 270 y 2200 m.

Fenología. Florece desde noviembre hasta abril.

Iconografía. Sanzin, 1919: 119, fig. 23 (sub *Verbena aspera*); Roig, 1970: 146, fig. 72 (sub *Verbena aspera*); Botta, 1999: 164, fig. 120.

Observaciones. La variedad típica presenta el cáliz glanduloso, tubo de la corola apenas más largo que el cáliz y las hojas generalmente trisectas.

Los ejemplares provenientes de San Juan, Dpto. Iglesia presentan hojas hasta de 6 mm lat., sin embargo dado que el resto de caracteres coinciden con *Junellia aspera* se los incluye dentro de esta especie. De acuerdo con Botta, 1987: 242, la especie ha sido excluída de la flora peruana.

Se eligió como lectotipo de *Verbena ourostachya* el ejemplar *E. Wilczek 51*, depositado en G, por estar manuscrito el nombre de la especie y la referencia "sp. nov.", por el autor de la misma.

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Capayán, Sierra de Ambato, Quebrada y Cuesta de la Sébila, ruta 60, entre Chumbicha y el desvío hacia Pomán, *A. T. Hunziker 18390* (SI). **La Pampa**: Dpto.

Curacó, a 25 km de Puelches, Laguna Amarga y salares de Chadileufú, *Ruíz Leal 26046* (SI). **La Rioja**: Dpto. Felipe Varela, Parque de Talampaya, *A. T. Hunziker 11659* (SI). **Mendoza**: Dpto. Rivadavia, El Carrizal, *Múlgura 364* (SI). **Neuquén**: Dpto. Añelo, meseta al N de Mina Escondida (Auca Mahuida), *Correa 8662* (SI). **San Juan**: Dpto. Iglesia, ruta 150, 18 km al W de Las Flores, Valle de Pismanta, *J. H Hunziker 11622* (SI). **San Luis**: Dpto. Belgrano, Sierra de Las Quijadas, quebrada del Alambre, al W de San Antonio, ruta nac. 147, *A. T. Hunziker 16359* (SI). **Río Negro**: Dpto. Avellaneda, 35 km al E de Choele Choel, *Cabrera 18605* (SI).

35b. Junellia aspera var. **longidentata** (Moldenke) Múlgura & P. Peralta, comb. nov. Basónimo: *Junellia longidentata* Moldenke, Known Geogr. Distrib. Verb. Avicenn.: 77. 1942. *Verbena aspera* Gillies & Hook. ex Hook. var. *longidentata* (Moldenke) Botta, Darwiniana 25: 338. 1984. TIPO: Argentina. Tucumán: Amaicha, 2090 m, 29 dic. 1912, *L. Castillón 2460* (holotipo, NY; isotipo, LIL 31753!).

Ramas jóvenes sección tetrágona, hispídulas flexibles. Hojas generalmente homomorfas, enteras, de 1.3–2.5 × 0.3–0.8 cm, en los macroblastos, opuestas, brevemente pecioladas, y en los braquiblastos, fasciculadas, lámina membranácea, elíptica u obovada, ápice generalmente agudo, a veces mucronado, margen entero, pubescencia de pelos cortos y rígidos, dispuestos espaciadamente en ambas caras. Raquis, brácteas y cáliz, incano-vellosos. Tubo calicinal de 4.5–5 mm, dientes subiguales ca. 1.5–2 mm, ápice subulado. Corola de 9–10.5 mm, tubo de 6.5–8 mm, recurvo, glabro.

Cariología. $2n = 40$ (*Junellia longidentata*, Poggio et al., 1988: 542, 544, fig. 1C).

Distribución geográfica. Noroeste de Argentina, desde Jujuy hasta La Rioja (Fig. 1A). Habita en la zona alta de la provincia del Monte, llegando hasta los 3000 m en la Prepuna.

Fenología. Florece de diciembre a marzo.

Iconografía. Botta, 1984: 339, fig. 3 (sub *Verbena aspera* var. *longidentata*); Botta, 1993: 71, fig. 31 (sub *Junellia longidentata*).

Observaciones. Especie afín a *Junellia arequipensis* por el porte; se diferencia por presentar sección del tallo poligonal, cáliz estrigoso, con dientes triangulares, corola pardo-amarillenta, 2 a 3 veces más larga que el cáliz, y las alas del pericarpo pequeñas.

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Andalgalá, Andalgalá, *Jörgensen 1614* (SI); Dpto. Belén, Hualfin, *Sayago 600* (SI). **La Rioja**: Dpto. Gral. Sarmiento, entre el peñón y el Jagüe, aproximadamente a 11 km del primero, quebrada del río El Peñón, *Biurrun 4578* (SI). **Jujuy**: Dpto. Tilcara, Quebrada de Huasamayo, *Botta 137* (SI). **Salta**: Dpto. Cachi, recta de Tin-tin, *Kiesling 6411* (SI). **Tucumán**: s. loc., *Morello 1287* (SI).

36. Junellia cinerascens (Schauer) Botta, Darwiniana 29: 392. 1989. Basónimo: *Verbena cinerascens* Schauer in DC., Prodr. 11: 545. 1847. *Diostea cinerascens* (Schauer) Moldenke, Revista Sudamer. Bot. 5: 1. 1937. TIPO: Chile. IV Región, Coquimbo: 1829, *C. Gay s.n.* (lectotipo, designado por Botta (1989: 392), G!, G foto FM 7855 SI! [ejemplar a la derecha de la foto]; isotipos, F!, K!). Figura 11A–G.

Arbustos cinéreos, erguidos, 2 m alt.; ramas espinescentes, rígidas, sección tetragonal, canotomentosas, generalmente sin desarrollo de braquiblastos. Hojas homomorfas de 4–8(–15) × 1–5 mm, subsésiles, lámina ovada o elíptica-angosta, ápice agudo, base decurrente, pelos patentes y rígidos en ambas caras. Monobotrios plurifloros, espigas de 4–8 cm, alargadas; brácteas de 2–2.5 mm, ovadas, pelos adpresos en su superficie. Cáliz de 4.5–4.8 mm, con pelos blancos rígidos y adpresos, dientes de 1 mm, los adaxiales mayores, los restantes brevísimos; corola de 12–14 mm, blanca hasta rosada, superficie externa pubescente en la mitad superior del tubo y base de los lóbulos; superficie interna del tubo y fauce pubescente; par superior de estambres con apéndices conectivales exertos y claviformes, conectivo superando la longitud de las tecas; gineceo 7.5–8 mm, base del estilo no ensanchada, hundida profundamente entre las clusas. Clusas ca. 3.5 × 1.2 mm, ápice obtuso, cara dorsal parda, caras comisurales blancas papilosas.

Distribución geográfica. Hasta el momento sólo se ha coleccionado en Chile en las regiones IV y V, entre los 30°S y 33°S (Fig. 1B). Habita en faldeos y quebradas entre los 1000 y 1500 m, en la provincia fitogeográfica Chilena Central, dominio Andino-patagónico.

Fenología. Florece y fructifica en verano.

Observaciones. Esta especie se caracteriza por el estilo profundamente hundido en el ovario; este carácter se hace más pronunciado al desarrollarse el fruto. Es afín a *Junellia scoparia* que presenta sección del tallo poligonal, ausencia de apéndices conectivales y el estilo apenas inserto entre las clusas.

Citharexylon alpinum Poepp., es un nombre manuscrito en herbario, no publicado validamente. Fue citado por Walpers (1845: 78) como sinónimo de *Verbena scoparia* Gillies & Hook. ex Hook. y posteriormente por Schauer (1847: 545) como sinónimo de *V. cinerascens* Schauer. Moldenke (1960: 309) lo consideró en la sinonimia de *Diostea cinerascens*

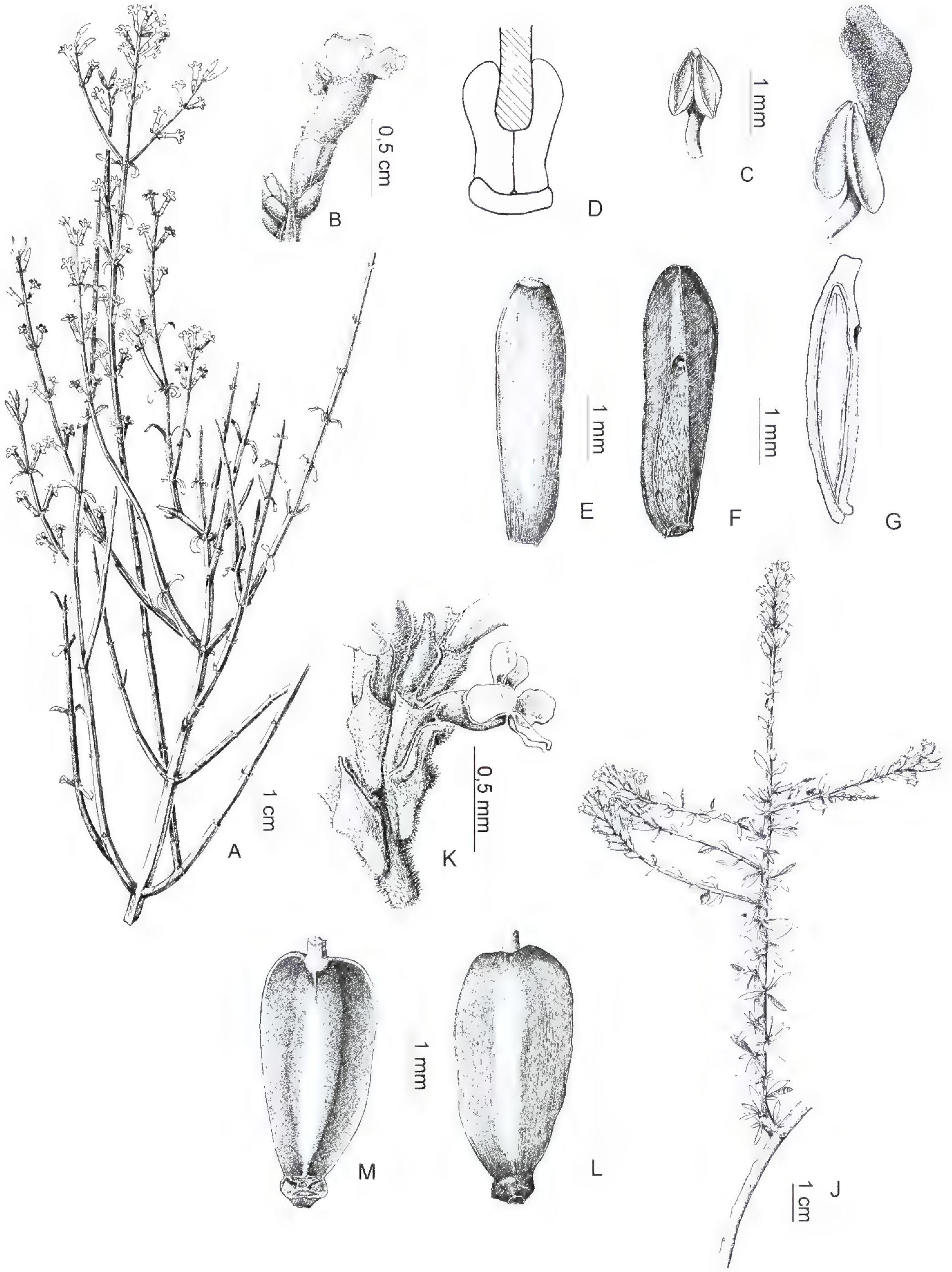


Figura 11. A–G. *Junellia cinerascens*. —A. Aspecto general. —B. Flor. —C. Anteras. —D. Esquema del corte longitudinal de ovario. —E. Fruto cara adaxial. —F. Fruto cara abaxial. —G. Corte longitudinal de fruto. A–G de *Marticorena 401*. J–M. *J. echegarayi*. —J. Aspecto general. —K. Inflorescencia. —L. Fruto vista adaxial. —M. Fruto vista abaxial. J–M de *Kiesling 3267*.

(Schauer) Moldenke y hace referencia a las fotos del citado ejemplar de Poeppig (FM 7855).

Material adicional examinado. CHILE. **IV Región**: Limarí, Estancia Cabrería, faldeos, *Jiles 1535* (CONC). **V Región**: Retorca, Los Andes por Río Colorado, 2 dic. 1929, *Behn s.n.*, CONC 22209 (CONC).

37. Junellia echegarayi (Hieron.) Moldenke, Lilloa 5: 395. 1940. Basónimo: *Verbena echegarayi*

Hieron., Bol. Acad. Nac. Ci. 4: 66. 1881. TIPO: Argentina. San Juan: Leoncito, ene. 1876, *S. Echegaray s.n.* (holotipo, CORD!). Figura 11J–M.

Junellia echegarayi var. *cordifolia* Moldenke, Phytologia 2: 465. 1948, syn. nov. TIPO: Argentina. Mendoza, Las Heras: Uspallata, 2300 m, 21 dic. 1937, *K. Grandjot s.n.*, Hb. Ruíz Leal 4714 (holotipo, MERL!).

Junellia echegarayi var. *puberulenta* Moldenke, Phytologia 2: 466. 1948, syn. nov. TIPO: Argentina. Mendoza, Las Heras: Uspallata, 21 dic. 1937, *K. Grandjot s.n.*, Herb. Ruíz Leal 4713 (holotipo, MERL!).

Arbusto de 0.40–1.20 m de alt.; tallos jóvenes de sección poligonal o circular, pubérulos, al envejecer glabrescentes y espinescentes; corteza pardo amarillenta, estriada longitudinalmente. Hojas dimorfas, enteras, las de los macroblastos opuestas, de 6–8(–12) × 1.5–4 mm, subpecioladas, recurvas, ovadas, ápice agudo, base obtusa o cordiforme, margen algo revoluto, cara adaxial algo escabrosa y abaxial híspida; las de los braquiblastos sésiles, imbricadas, de 4–12 × 1.5–3 mm, espatuladas, ápice obtuso, base atenuada, margen algo revoluto y pubescencia similar a las hojas de los macroblastos. Monobotrios en espigas plurifloras, de 2.5–5(–6) cm, flores subverticiladas; brácteas de 3–5 × 2–3 mm, ovadas a veces rombiformes, ápice agudo o acuminado, pilosas en el margen. Cáliz de 5.5–6 mm, hispídulo; dientes muy breves, triangulares, ápice rígido, subiguales, el abaxial de 0.5 mm; corola de 9–11.5 mm, lila o blanca, glabra en el exterior o con pocos pelos esparcidos, pubescente en el interior, fauce glabra, ápice obtuso; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 6 mm, base del estilo no ensanchada. Clusas de 4–4.1 × 2–2.2 mm, glabras, con el pericarpio expandido lateralmente formando dos alas conspicuas, ápice obtuso, base angostada, cara dorsal lisa, pardo oscuro, caras comisurales papilosas, blancas.

Distribución geográfica. Argentina en San Juan y Mendoza; citada para Perú por Macbride (1960: 620), en base al ejemplar *Weberbauer 1398*, no se ha tenido acceso a este ejemplar, ni tampoco a otros materiales, que avalen efectivamente la presencia de la especie en Perú (Fig. 1C). Crece en el límite entre la provincia fitogeográfica del Monte y la Prepuna, entre los 1900 y 3000 m, en planicies secas y arenosas.

Fenología. Florece y fructifica en verano.

Iconografía. Sanzin, 1919: 120, fig. 24 (sub *Verbena echegarayi*).

Observaciones. Especie muy afín a *Junellia aspera*, de la que se diferencia principalmente por los dientes del cáliz subulados y de mayor tamaño y la pubescencia del cáliz glandulosa.

Las variedades fundadas por Moldenke se consideran sinónimos de la especie porque al observar mayor cantidad de ejemplares no se encontraron diferencias suficientes como para mantenerlas.

Material adicional examinado. ARGENTINA. **Mendoza**: Dpto. Las Heras, Paramillo de Uspallata, *Ruíz Leal 23294* (SI); Dpto. Tupungato, Tupungato, *Ruíz Leal 2913* (SI). **San Juan**: Dpto. Iglesia, camino al Valle del Cura, llanos del Romo, *Kiesling 3267* (SI).

38. Junellia ligustrina (Lag.) Moldenke.

38a. Junellia ligustrina (Lag.) Moldenke var. **ligustrina**, Phytologia 2(11): 466. 1948. Basónimo: *Verbena ligustrina* Lag., Gen. Sp. Pl. 18. 1816. *Lippia ligustrina* (Lag.) Britton, Trans. New York Acad. Sci. 9: 181. 1890. *Aloysia ligustrina* (Lag.) Small, Fl. South. U.S. 1013. 1903. TIPO: Argentina. Santa Cruz, Deseado: a 107 km al S del límite con Chubut, ruta Nac. 3, 25 feb. 1990, *M. N. Correa et al. 10256* (neotipo, aquí designado, SI!). Figura 10E.

Arbustos erguidos, de 1–1.5 m alt. Ramas rígidas de sección tetragonal. Hojas homomorfas, opuestas, a veces subalternas, de 1–3 × 0.2–1.2 cm, enteras, coriáceas, elípticas u oblongas, ápice obtuso, mucronado, base obtusa o atenuada, escabrosas. Monobotrios en espigas alargadas, plurifloras de (3–)4.5–9 cm, flores perfumadas; brácteas de 4 mm, angostamente ovadas, pubescentes. Cáliz de 5–6 mm, dientes triangulares, subiguales, de 1 mm, con pubescencia adpresa; corola amarillenta con fauce rojiza, glabra, de 10–16 mm, tubo recurvo, glabro en el exterior, pubescente en el interior; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 10–14 mm, estilo con la base no ensanchada. Clusas de 4–4.5 mm, glabras, pericarpio prolongado lateralmente formando dos alas conspicuas; cara dorsal pardo-oscura, caras comisurales blancas papilosas.

Cariología. $2n = 20$ (sub *Verbena ligustrina*, Covas & Hunziker, 1954: 251); (*Junellia ligustrina*, Botta & Brandham, 1993: 146, fig. 1D).

Nombre vulgar. "Verbena" (Soriano, 1956a: 326).

Distribución geográfica. Habita en Argentina, en Neuquén, Chubut y Santa Cruz; crece la provincia fitogeográfica Patagónica desde el nivel del mar, hasta los 2000 m formando parte de las estepas arbustivas áridas y semiáridas (Fig. 1D). Fue citada para Perú; no se ha observado material que lo avale (Pool, 1993: 1171).

Fenología. Florece en verano.

Iconografía. Botta, 1999: 171, figs. 127, 173.

Observaciones. Comparte con *Junellia aspera* y *J. echegarayi* los frutos alados lateralmente. Se diferencia de ambas porque presentan las hojas membranáceas; *J. aspera* además presenta las hojas de los macroblastos tripartidas.

Como se expresó en otros casos los ejemplares estudiados por Lagasca han sido destruidos, por lo tanto se eligió un ejemplar que coincide con los caracteres de la especie.

Lippia ligustrina (Lag.) Kuntze (Revis. Gen. Pl. 3(2): 252. 1898) es un nombre ilegítimo por ser un homónimo posterior.

Material adicional examinado. ARGENTINA: **Chubut**: Dpto. Biedma, 8 km S de Puerto Madryn, ruta prov. 1, *Correa 10161* (SI). **Neuquén**: Dpto. Catan Lil, La Negra, *Schajovskoy s.n.*, SI 25628 (SI). **Santa Cruz**: Dpto. Deseado, Valle del río Deseado, ruta 277, *Soriano 5051* (SI).

38b. Junellia ligustrina var. **lorentzii** (Niederl. ex Hieron.) Moldenke, Phytologia 47: 222. 1980. Basónimo: *Verbena lorentzii* Niederl. ex Hieron., Bol. Acad. Nac. Ci. Córdoba 3(4): 370. 1880. *Junellia lorentzii* (Niederl. ex Hieron.) Moldenke, Lilloa 5: 397. 1940. TIPO: Argentina. Río Negro, set.-nov., 1874, *C. Berg 100* (lectotipo, aquí designado, CORD!). Figura 10F.

Verbena inconcinna Briq., Annuaire Conserv. Jard. Bot. Genève 4(2): 19. 1900. TIPO: Argentina. Mendoza: Cerroyo Manga, entrée de la vallée de l'Atuel, *E. Wilczek 49* (holotipo, G!; isotipos, MO no visto, SI!, B foto FM 24688 SI!).

Verbena alatocarpa Tronc., Darwiniana 8: 485. 1949. Nombre reemplazado: *Junellia chubutensis* Moldenke, Phytologia 2: 465. 1948, nom. illeg., non *Junellia chubutensis* (Speg.) Moldenke, Lilloa 5: 394. 1940. *Junellia alatocarpa* (Tronc.) Moldenke, Phytologia 3: 167. 1949. TIPO: Argentina. Chubut: Puerto Madryn, 24 oct. 1945, *C. O'Donell 1945* (holotipo, NY!; isotipos, LIL!, SI!).

Difiere de la variedad típica por las hojas en general más pequeñas, las inflorescencias menores, 1.5–3(–6) cm y el cáliz con pubescencia híspida o hispídula.

Cariología. $2n = 20$ (sub *Verbena inconcinna*, Rahn, 1960: 123).

Distribución geográfica. Crece en Argentina desde el sur de Mendoza y Buenos Aires hasta Santa Cruz, en la provincia fitogeográfica Patagónica, desde el nivel del mar hasta los 2000 m (Fig. 1D).

Fenología. Florece desde octubre hasta febrero.

Iconografía. Lorentz y Niederlein, 1881: tab. XII, I, figs. 2–6 (sub *Verbena lorentzii* Niederl.).

Observaciones. Se eligió como lectotipo de *Verbena lorentzii*, el ejemplar de C. Berg, ya que la publicación de Hieronymus, se basa en materiales de dicho coleccionista y los originales se encuentran depositados en CORD. Existe una foto: B foto FM 17428, que podría corresponder a la descripción del homónimo posterior *Verbena lorentzii* Niederl. (Lorentz & Niederlein, 1881: 264, tab. XII, I, figs. 2–6).

Material adicional examinado. ARGENTINA. **Buenos Aires**: Dpto. Carmen de Patagones, Médano Comody, *Miccio Peralta 30* (SI). **Chubut**: Dpto. Biedma, Puerto Madryn, *Soriano 2717* (SI). **La Pampa**: Dpto. Chical Co, Aguas de Torres, *Adler s.n.* (SI); Dpto. Lihuel Calel, Sierra Lihuel Calel, *Cabrera 19437* (SI). **Mendoza**: Dpto. Malargüe, Sierras de Chachahuén, Cerro de Ureta, *Prina 1454* (SI). **Neuquén**: Dpto. Catán Lil, Ruta 40, entre La Negra y Catán Lil, *Gentili 831* (SI). **Río Negro**: Dpto. El Cuy, a 70 km del Cerro Policia, *Correa 4750* (SI). **Santa Cruz**: Dpto. Lago Argentino, ruta 40, 16 km N de Paso Biggeri, sobre río La Leona, *Boelcke 12654* (SI).

39. Junellia scoparia (Gillies & Hook. ex Hook.) Botta, Darwiniana 29: 392. 1989. Basónimo: *Verbena scoparia* Gillies & Hook. ex Hook., Bot. Misc. 1: 161. 1829. *Diostea scoparia* (Gillies & Hook. ex Hook.) Miers, Trans. Linn. Soc. London 27: 104. 1869. TIPO: Argentina. Mendoza: Las Heras, shrub valley nr. Villavicencio, *J. Gillies s.n.* (lectotipo, designado por Botta (1989: 392), K!, K foto SI!). Figura 12A–G.

Lippia scirpea Phil., Anales Univ. Chile 21: 402. 1862. *Diostea scirpea* (Phil.) Miers, Trans. Linn. Soc. London 27: 107. 1869. TIPO: Argentina. Mendoza: Portezuelo del Portillo, 1861–1862, *W. Díaz s.n.*, SGO 42407 (lectotipo, designado por Acevedo de Vargas (1951: 42), SGO!; isotipo, SI!).

Lippia aphylla Phil., Anales Univ. Chile 90: 623. 1895. TIPO: Chile. Valparaíso: Campana de Quillota, *Aug. Borchers 1884*, SGO 54829 (lectotipo, designado por Acevedo de Vargas (1951: 44), SGO!).

Verbena scoparia Gillies & Hook. ex Hook. var. *puberula* Tronc., Dansk. Bot. Ark. 22(1): 109. 1963, syn. nov. *Diostea scoparia* var. *puberula* (Tronc.) Moldenke, Phytologia 19: 319. 1970. *Junellia scoparia* var. *puberula* (Tronc.) Botta, Hickenia 2: 128. 1995. TIPO: Argentina. Mendoza, San Rafael: Cuesta de los terneros, 30 km SW de San Rafael, 34°S, 1200 m, 22 nov. 1955, *T. Böcher 1143* (holotipo, SI!).

Arbustos erguidos, de 0.90–1.20 m de alt., subáfilos; ramas opuestas, rígidas, de sección poligonal, espiniformes, sin desarrollo de braquiblastos. Hojas de 5–6 × 3 mm, opuestas, sésiles, enteras, caedizas, lámina ovada o linear, ápice obtuso y base decurrente. Monobotrios o dibotrios plurifloros, en espigas de 2.5–5 cm, alargadas, raquis de las florescencias glabro o pubérulo; brácteas de 1.5–3 mm, ovadas o excepcionalmente redondeadas, subuladas, generalmente ciliadas en el margen, superficie adaxial glabra o pubérula; flores perfumadas. Cáliz de 4–5.5 mm, glabro o pubérulo, dientes desiguales, triangulares los abaxiales de 0.5 mm, los adaxiales

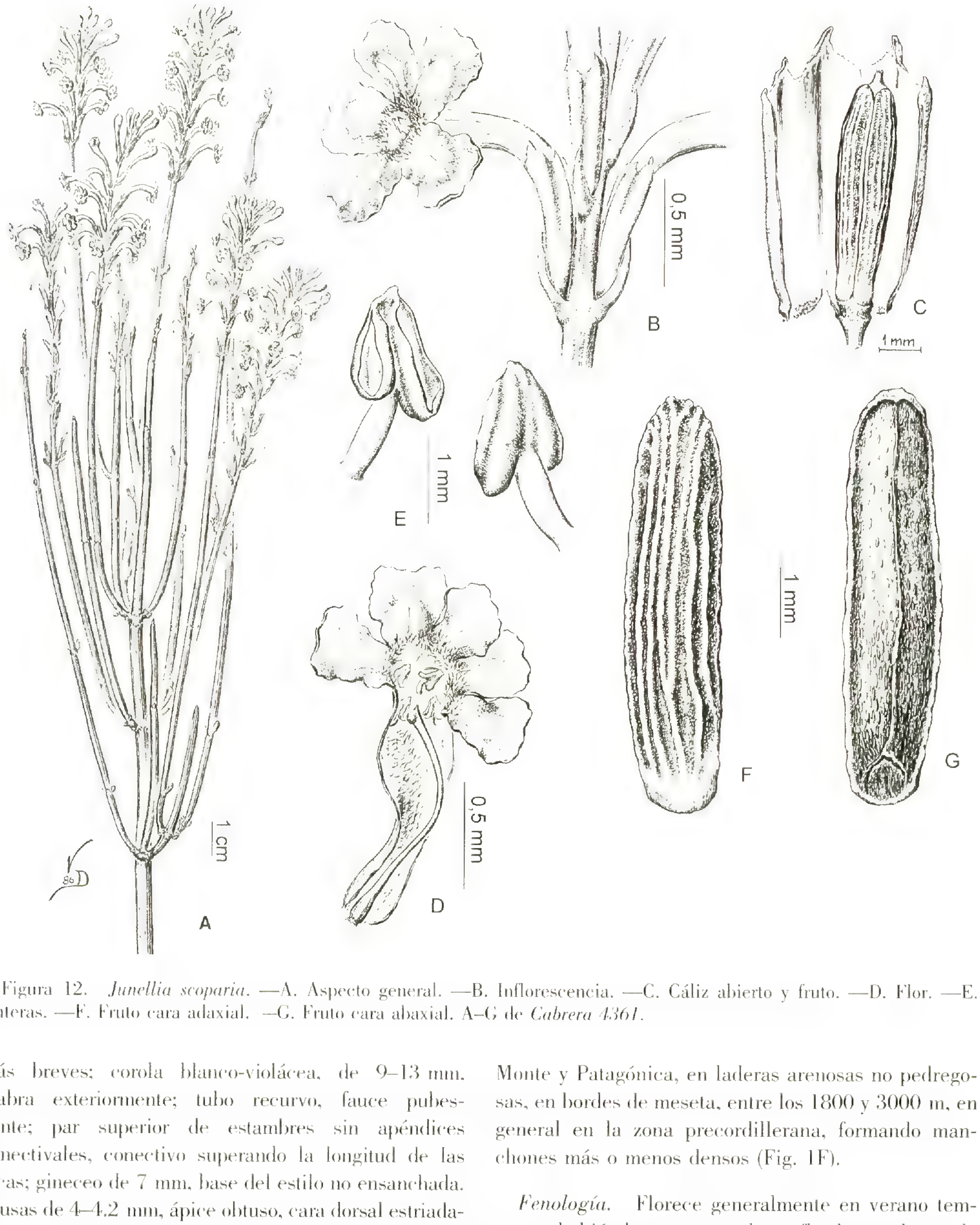


Figura 12. *Junellia scoparia*. —A. Aspecto general. —B. Inflorescencia. —C. Cáliz abierto y fruto. —D. Flor. —E. Anteras. —F. Fruto cara adaxial. —G. Fruto cara abaxial. A–G de *Cabrera 4361*.

más breves; corola blanco-violácea, de 9–13 mm, glabra exteriormente; tubo recurvo, fauce pubescente; par superior de estambres sin apéndices conectivales, conectivo superando la longitud de las tecas; gineceo de 7 mm, base del estilo no ensanchada. Clusas de 4–4.2 mm, ápice obtuso, cara dorsal estriada-reticulada, pardo-oscura, caras comisurales blancas, papilosas.

Cariología. $2n = 20$ (sub *Diostea scoparia*, Covas & Schnack, 1946: 155).

Nombre vulgar. "Clavillo del campo", "escobilla del campo" (Acevedo de Vargas, 1951: 44); "solupe negro" (Ruíz Leal, 1972: 228).

Distribución geográfica. Chile, regiones IV, V y Región Metropolitana y Argentina desde Catamarca hasta Río Negro, en las provincias fitogeográficas del Monte y Patagónica, en laderas arenosas no pedregosas, en bordes de meseta, entre los 1800 y 3000 m, en general en la zona precordillerana, formando manchones más o menos densos (Fig. 1F).

Fenología. Florece generalmente en verano temprano, habiéndose encontrado en flor hasta el mes de abril.

Iconografía. Hooker, 1829, Bot. Misc. 1: tab. 47 (sub *Verbena scoparia*); Sanzin, 1919: 123, fig. 26 (sub *Verbena scoparia*); Acevedo de Vargas, 1954: 43, fig. 1A (sub *Lippia aphylla*), C (sub *Lippia scirpea*), D (sub *Verbena scoparia*), E (sub *Verbena scoparia* var. *aphylla*); Troncoso, 1957: 169, fig. 2, Vs corte anatómico de tallo (sub *Verbena scoparia*); Ruíz Leal, 1972: 227, lám. 70, 265 (sub *Verbena scoparia*); Botta, 1999: 178, 180, fig. 132 (sub *Junellia scoparia*).

Observaciones. Los ejemplares de esta especie se caracterizan por sus tallos aparentemente áfilos y que ennegrecen al secarse. Se diferencia de *Junellia cinerascens* que presenta tallos de sección tetragonal y base del estilo profundamente hundido entre las clusas.

De acuerdo a la observación del material es muy díficil poder establecer los límites entre las diferentes variedades, ya que por un lado la pubescencia es muy variable en densidad y por otra sólo se encuentra algún ejemplar con brácteas más subovadas o suborbiculares, como para justificar la revalidación de la variedad definida por Acevedo de Vargas.

En el caso de *Lippia aphylla*, Acevedo de Vargas (1951: 44) escribe: "Valparaíso: Campana de Quillota, *Aug. Borchers 1884* (Clastotypus et typus de *Lippia aphylla*: SGO 42424 et 54829)". Posteriormente la misma autora (1954: 42) explica que el ejemplar SGO 54829, está "en perfecto estado y que permite distinguir bien sus caracteres". En el material estudiado, vuelve a citarlos, pero invirtiendo el orden de los números de los ejemplares ("Typus y Clastotypus SGO 42424 y 54829"). De acuerdo con nuestras observaciones efectivamente el ejemplar SGO 54829, es el más completo, por lo cual se considera efectiva la lectotipificación de Acevedo.

Verbena scoparia Gillies & Hook. ex Hook. var. *aphylla* (Phil.) Acevedo de Vargas (Acevedo de Vargas, 1954: 45) es un nombre ilegítimo, de acuerdo al Art. 33.3 del *Código Internacional de Nomenclatura Botánica* (Greuter et al., 2000).

Verbena scoparia Tausch (Flora 19(2): 390. 1836) es un nombre ilegítimo por ser un homónimo posterior.

Moldenke describió *Diostea scoparia* var. *subulata* Moldenke (Phytologia 44: 123. 1979). El material citado por Moldenke, pertenece a *Neosparton aphyllum* (Gillies & Hook. ex Hook.) Kuntze; por esta razón este taxón no se considera en la sinonimia de *Junellia scoparia*.

Material adicional examinado. ARGENTINA. **Catamarca**: Dpto. Andalgalá, La Plaza, *Jörgensen 1403* (BA, K, SI). **La Rioja**: Dpto. Chilecito, estafeta Santa Florentina, camino a la Mina de Oro, *von Rentzell s.n.*, SI 19233 (SI). **Mendoza**: Dpto. Las Heras, Quebrada del Toro, *Ruíz Leal 25904* (SI). **Neuquén**: Dpto. Aluminé, entre piedra Gaucha y Aluminé, *Cabrera 32952* (SI). **Río Negro**: Dpto. Picaniyeu, a 6 km de Pilcaniyeu, camino a Bariloche, *Nicora 3684* (SI). **San Juan**: Dpto. Angaco, Sierra de Pie de Palo, camino al Mogote de los Corralitos, *Kiesling 4810* (SI). CHILE. **IV Región**: Coquimbo, Illapel, Cerro Curimahuida, *Goodspeed 16692* (SI). **V Región**: Aconcagua, llano de Juncalillo, *Ricardi 2910* (SI); Valparaíso, Cerro La Campana, *Hutchinson 42* (K). **Región Metropolitana**: Santiago, Cordillera de Santiago, Cerro Cortadera, *Werdermann 488* (K, SI).

TAXONES DUDOSOS

Junellia hayekii Moldenke, Lilloa 5: 396. 1940. Nombre reemplazado: *Verbena aretioides* Hayek, Bot. Jahrb. 42: 163. 1908, hom. illeg. TIPO: Perú. Iter a Tacora ad Pomarape, 4200–4400 m s.m., oct. 1876, *Stübel 100b* (holotipo, B†, B foto FM 17402 SI!).

Para conocer si existía el material citado por Hayek, se consultó a los siguientes herbarios: B, BR, C, CAS, F, G, GB, GH, GOET, H, K, LE, NY, PH, S, TEX, US, W, WRSL, WU, y en ninguno de ellos existe el material. El siguiente ejemplar: PERÚ. Entre Puno y Arequipa, mar. 1943, *Sanderman 3855* (K), que fue citado bajo este nombre, se considera aquí, que es *Junellia minima*, especie citada para la misma región geográfica.

Verbena polycnemoides Lag., Gen. Sp. Pl. 18. 1816. TIPO: "in Bonaria".

Como se expresó anteriormente los materiales de Lagasca fueron destruidos, en este caso la descripción es poco clara y además en la "planicie bonaria", no crece *Junellia*.

Verbena spartioides Turcz., Bull. Soc. Imp. Naturalistes Moscou 36(3): 195. 1863. TIPO: Chile. Cumbre, *Mac Rae 43* (no localizado).

No fue posible localizar el ejemplar tipo, por lo que se considera a esta especie como un taxón dudoso. De acuerdo con la descripción original por el tallo tetrágono, cinéreo y hojas pequeñas, podría tratarse de un nombre sinónimo de *Junellia cinerascens*.

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Apéndice 1. Lista de las especies aceptadas del género *Junellia*.

34. *J. arequipensis* (Botta) Botta
2. *J. aretioides* (R. E. Fr.) Moldenke
29. *J. asparagoides* (Gillies & Hook. ex Hook.) Moldenke
35a. *J. aspera* (Gillies & Hook. ex Hook.) Moldenke var. *aspera*
35b. *J. aspera* var. *longidentata* (Moldenke) Múlgura & P. Peralta
3. *J. azorelloides* (Speg.) Moldenke
14a. *J. bisulcata* (Hayek) Moldenke var. *bisulcata*
14b. *J. bisulcata* var. *campestris* (Griseb.) Botta
15. *J. bryoides* (Phil.) Moldenke
16. *J. caespitosa* (Gillies & Hook. ex Hook.) Moldenke
30. *J. cedroides* (Sandwith) Moldenke
36. *J. cinerascens* (Schauer) Botta
4. *J. congesta* (Tronc.) Moldenke
17. *J. connatibracteata* (Kuntze) Moldenke
5a. *J. digitata* (Phil.) Moldenke var. *digitata*
5b. *J. digitata* var. *integerrima* (Botta) Botta
37. *J. echegarayi* (Hieron.) Moldenke
18. *J. erinacea* (Gillies & Hook. ex Hook.) Moldenke
31. *J. hystrix* (Phil.) Moldenke
19. *J. juniperina* (Lag.) Moldenke
25. *J. lavandulaefolia* (Phil.) Moldenke
38a. *J. ligustrina* (Lag.) Moldenke var. *ligustrina*
38b. *J. ligustrina* var. *lorentzii* (Niederl. ex Hieron.) Moldenke
1. *J. micrantha* (Phil.) Moldenke
6. *J. minima* (Meyen) Moldenke
20. *J. odonellii* Moldenke
7. *J. patagonica* (Speg.) Moldenke
26. *J. pseudojuncea* (Gay) Moldenke
39. *J. scoparia* (Gillies & Hook. ex Hook.) Botta
27a. *J. selaginoides* (Kunth ex Walp.) Moldenke var. *selaginoides*
27b. *J. selaginoides* var. *illapelina* (Phil.) Botta
21. *J. seriphioides* (Gillies & Hook. ex Hook.) Moldenke
8. *J. silvestrii* (Speg.) Moldenke
28a. *J. spathulata* (Gillies & Hook. ex Hook.) Moldenke var. *spathulata*
28b. *J. spathulata* var. *glauca* (Gillies & Hook. ex Hook.) Botta
22. *J. spissa* (Sandwith) Moldenke
9. *J. succulentifolia* (Kuntze) Moldenke
32. *J. tetragonocalyx* (Tronc.) Moldenke
10. *J. thymifolia* (Lag.) Moldenke
23a. *J. toninii* (Kuntze) Moldenke var. *toninii*
23b. *J. toninii* var. *mulinoides* (Speg.) P. Peralta & Múlgura
11. *J. tridactylites* (Lag.) Moldenke
33. *J. tridens* (Lag.) Moldenke
12. *J. trifurcata* (Phil.) Moldenke
24. *J. ulicina* (Phil.) Moldenke
13. *J. uniflora* (Phil.) Moldenke

Apéndice 2. Índice de colecciones examinadas. Cada espécimen es citado por el nombre del primer colector en el caso en que otros coleccionistas participen de la colección. En el caso de no poseer número de colección se indica el número del herbario en que se encuentra depositado o en su defecto sólo el herbario. Se indica entre paréntesis el número de orden de la especie a que corresponde (ver Apéndice 1).

Abadie 748(9), 986(20b), 1152(11); Adler s.n.(35a), s.n.(17); Ager 580(33), 581(18); Aguirre 455(21); Albornoz 11(21); Allende 222(39); Ambrosetti 1591(35a), 1257(18), 1291(18), BA 27/887(21), 3133(28b); Ameghino 19(10) 32(7), 78(3), 89(24), 190(33), s.n.(20a), s.n.(1), s.n.(24), s.n.(24), aest. 1899(24), LP 1135(24), s.n. LP 10007(11), s.n. LP 10008(11), s.n. LP 10009(11), s.n. LP 12990(7), s.n. LP 12995(7), s.n. LP 13000(24), s.n. LP 13001(24); Amman 114(17), 117(20b); Anchorena s.n.(9); Ancibor 1(6), 31(21), 48(2), 51(2), 102(7), s.n. BAA 4453(33), s.n. BAB 4458(20a), s.n. BAB 4480(23p.p.), s.n. BAB 4480(8p.p.), s.n. BAB 4630(7), s.n. BAB 5788(24), s.n. BAB 90128(28b), s.n. BAB 90130(17), s.n. BAB 90141(16), s.n. BAB 90212(20a), s.n. BAB 90222(28b), s.n. BAB 90250(38b), BAB 90263(30), s.n. BAB 90289(17); Anderson 1664(21); Andrada s.n.(37); Angelleli 8(28b); Anliot 6007(33), SGO 78425(33); Anz 4(21); Apochian 58(37), 183(37), 200(18), 195(21), 246(21), 248(37); Arancio 379(5a), 657(29), 662(13), 10130(31), 10139(31), 10147(31), 10292(31), 10319(31), 10332(31), 10355(31), 10655(31), 94183(26); Araque s.n. LIL 1021(16), 1084(19), 1130(19); Arenas 84(34), s.n. BACF 954(29), s.n. BACP 1723(6); Arriaga s.n. BA 75895(21), BA 75881(21); Arroyo 16(20b), 66(17), 67(10), 101(20a), 114(10), 116(20a), 130(33), 131(20a), 145(11) 182(20a), 194(7), 212(10), 216(20a), 256(7), 283(38b), 329(31), 331(20), 332(20), 337(1), 341(20a), 355(10), 376(38a), 380(5b), 415(38b), 417(31), 418(20a), 470(31), 478(38b), 479(20a), 500(7), 515(5b), 563(6), 577(19), 831(19), 842(34), 847(19), 1306(31), 2589(6), 81273(13), 81603(13), 81296(39), s.n. TBPA 227(33), s.n. TBPA 262(33), s.n. TBPA 2096(8), s.n. TBPA 2178(8), s.n. TBPA 2188(33), s.n. TBPA 2187(33), s.n. TBPA 2455(20), s.n. TBPA 2465(3), s.n. TBPA 2760, s.n. TBPA 2583(3); Asensi s.n.(7); Asp 125(9), s.n. BAB 64416456(9); Asplund 5882(21); Aubert de la Rüe s.n.(33); Ayarde 350(5a).

BA 34771(7); BAA 11625(17), BAA 12577(21), BAA 16518(3), BAA 16518(3), BAA 17794(35a), BAA 17795(17), BAA 18527(20b), BAA 18546(20), BAA 18548(16), BAA 18611(17), BAA 18650(20a), BAA 18817(11), BAA 18821(11), BAA 24780(20b), BAA 24781(1), 24787(18), BAA 25508(9); BAB 9865(35a), BAB 9882(35a), 90259(38b); Baer s.n. LIL 31617(19); Baeza 466(31), 649(31); Balegno 254(14a), 1180(14a); Ball s.n.(5a), 5973(29), 6000(6); Barkley 19 Ar 743(35a), 19 Ar 758(6), 19 Ar 852(19), 9232(6); Bartlett 19361(35a), 19363(35a), 19426(19), 19429(35a), 19434(21), 19448(35a), 19474(21), 19518(17), 19935(21), 20482(14b); Bártoli 9/03(9), 21(20a), 25(20a), 25/02–2(20b), 26/03(20a), 47/03(32), 50/02–2(20a), 55/03(9), 58/02–2(38b), 52/02 BAA 24787(20a), 65/02–2(10), 69/02–2(32), 114/02–2(38b); Barros s.n.(27b), 1377(27a), s.n.(36), 2233(27b), 2233(28b); Bastión 602(14a); Baumann 61(5a); Bayer 2019(2); Beaufilo

338(33), 603(33); Beck 2376(6), 2377(6), 2124(6), 3980(6), 11755(21), 11775(6), 11795(21) 14110(6), 22073(14a), 22116(14a), 22376(6); Beeskow 106(20a), 432(11), 932(20b), 953(9), 1001(32), 1018(20b), 1023(38b), 1033(32), 1052(1), 1091(9), 1253(17), 1272(33), 1287(10), 1302(16), 1309(17), 2265(17), 3091(3), 3113(20a); Behn s.n.(15), s.n. (27a), s.n.(27b), s.n.(19), s.n.(36), CONC 8274(36), s.n. CONC 23739(31), CONC 22209, CONC 28603(36), s.n.(31), s.n.(28b); Belfiori s.n. LIL 31761(21); Belmonte 14(31), 127(31), 211(19), 214(19), 20038(6); Benh s.n. CONC 22217(12); Benove 263(11), 265(10); Beorchia 7(37), 14(13), 65(29); Berg 100(38b)(paratipo), 152(21); Bertiller 7534(38a), 17(38a), 797(38b), 891(20b), 899(20b), 1074(8), 1087(20b), 1089(21), 1111(21), 1358(38b), 1671(38b), 1876(10), 1877(10), 1878(10), 1903(33), 1912(20b), 1913(20b), 2261(38a), 2266(38a); Bettfreund 12335(11); Biganzoli 1169(28a), 1198(21); Birabén 2(10), 16(10), 32(38b), 68(10), 96(33), 120(33), 133(10), 138(38a), 154(33), 162(10), 177(33), 179(33), 201(20b), 368(21), 480(38b), 495(33), 519(10), 656(1), 763(21), 775(21), 1183(21), 3048(21), 3050(28a), 3062(29), 3075(29); Biurrun 1262(35a), 1274(35a), 1555(6), 4578(35b), 4951(21), 5013(35a), 5176(21), 5201(5a), 5488(21), 5591(5a), 5679(37), 5711(29), 5861(37), 5895(16), 5936(37), 7091(29), 7695 bis(21); Blake 28A(11), 65(10), 75A(33), 202(11), 370(33), 380(10), 421 A(10), 454(38a), 455(38a); Böcher 672(17), 732(38b), 800(16), 921(29), 953(16), 954(17), 1033(16), 1062(18), 1087(35a), 1113(39), 1180(1), 1238(28b), 1397(17), 1412(17), 1418(28a), 1508(1), 1510(28a), 1538(11), 1574(28b), 1623(17), 1845(9), 1874(13), 2068(17), 2069(19), 2113(19), 2159(16), 2208(37); Bodenbender s.n. SI 3395(11b), BA 23699(37); Boelcke 1143(39), 3630(9), 4010(35a), 4013(21), 4052(19), 4057(29), 4071(28b), 4211(28b), 4492(9), 4460(7), 4474(39), 6309(9), 6451(7), 7581(9), 7582(20), 7591(39), 8053(21), 9865(37), 9882(37), 9941(18), 9950(19), 9965(39), 10006(13), 10058(13), 10083(19), 10176(21), 10184(21), 10220(28a), 10259(38b), 10326(24), 10442(9), 10689(28b), 10750(11), 10777(11), 11111(28a), 11160(1), 11202(28a), 11283(13), 11350(21), 11377(11), 11574(21), 11660(24), 11689(28b), 12109(20b), 12140(33), 12362(33), 12370(8), 12372(20), 12506(11), 12654(38b), 12764(20a), 12787(10), 12835(38b), 13537(28b), 13688(28a), 13748(11), 11184(11), 15009(11), 15013(33), 15325(11), 15362(1), 15358(24), 15370(33), 15361(20a), 15438(21), 15569(39), 15572(29), 15619(16), 15624(29), 15650(21), 15673(35a), 15674(11), 15700(16), 15706(11), 15727(38b), 15788(29), 15821(17), 15824(11), 15880(20a), 15889(29), 16074(10), 16080(33), 16149(20a), 16225(20a), 16229(1), 16244(10), 16252(20a), 1625(20b), 16280(33), 16598(38b), 16732(10), 19325(11), s.n. BAB 90372(19), BAB 90373(19), s.n. TBPA 619(33); Bohi 217(29); Bonarelli s.n. BA 23729(33); s.n. BA 23728(33); Bonifacino 684(38b), 690(17), 696(32), 698(11), 702(20b), 705(1), 706(10), 707(20b), 731(7), 760(33), 761(21), 765(7), 786(10); Borchers 1884(39); Borge 180(33); Botta 136(29), 137(35b), 138(20b), 315(35b), 139(30), 356(2), 381(14a), 382(14a), 383(14a), 385(19), 406(20b), 407(20b), 410(21), 411(20b), 421(20b), 428(17), 431(20a), 433(17), 435(20b), 437(20b), 439(17), 441(17), 443(20a), 449(20a), 452(1), 454(33), 462(1), 463(33), 464(20a), 465(24), 466(10), 471(10), 472(10), 474(20a), 476(20a), 478(20a), 479(20a), 481b(20a), 481(33), 482(10), 483(11), 485(11), 486 b(20a), 487(11), 488(33), 501(11), 506(20), 507(20), 512(11), 514(11), 515(11), 517(11), 519(11), 522(20a), 523(20a), 525(11), 526(10), 528(10), 529(11), 530(10), 531(10), 532p.p.(10), 532p.p.(20a), 534(10), 539(20a), 541(20a), 542(10), 543(20a), 544(11), 545(20a), 547(10), 549(10), 550(1), 551(10), 553(10), 557(11), 559(11), 561(11), 562(17), s.n. SI 26674(22), s.n. SI 26673(20b), s.n. SI 26676(17); Bragadín 20339(19); Bridarolli 2207(9), 4321(21); Bridges s.n.(39), s.n.(27a), s.n.(28b), 458(28a), 1220(28a), 1353(27a); Bruch s.n. LP 8325(19); Brun 763(32), 888(21), 889(20b), 2379(10), 2421(11), 2589(11), 2774(11); Buchtien s.n.(6), s.n.(39), 131(6), 932(6), US 1177977(39); Budín 16(29), 7411(6), 7455(35b), 7487(29), s.n. SI 1496(6), s.n. BA 31/911(6); Burkart 2149(21), 2152(35a), 7455(35b), 8392(21), 8415(39), 14855(1), 15864(21), 15927(35a), 15950(21), 19220(17), 20110(14b), 20784(14b), 22065(14a), 22071(19), 26513(9), 29642(14b), s.n. SI 11728(29), SI 11986(29), s.n. SI 14247(17), SI 14248(28b), s.n. SI 11253(28a), SI 14254(28a), SI 14255(28a), SI 14256(28a), s.n. SI 14257(39), SI 14259(38b), SI 14260(38b), SI 14261(16), SI 14263(28b), SI 14264(29), SI 14265.(28a), SI 14267(29), s.n. SI 14268(1), SI 14275(28b), SI 14279(24), s.n. SI 14278(38b), SI 14280(21), SI 14851(38b), SI 14882(17), s.n. SI 22071(21); Burkart, S. 1268(20a); Burmeister 12(11), 23(33), 45(11), 103(11), 113(33), 144(20), 3444(11), 6527(1), 6436(20a), 6437(17), 6439(11), 6526(17), 6529(38b), 6532(33), 6533(20b), 6534(7), 6535(20a), 11882(20b), 11898(11), 36530(38a), s.n. BAB 11921(33), s.n. SI 3446(33).

C. E. 516(27a); Cabrera 18(17), 22(33), 30(21), 38(38b), 115(5a), 121(38b), 129(2), 981(21), 1913(21), 3137(19), 3378(38b), 4382(21), 4802(38b), 4833(10), 4845(17), 4846(17), 4847(17), 7677(29), 7913(21), 7932(35b), 8216(21), 8244(21), 8270(2), 8401(5b), 8788(5a), 8952(5a), 9023(21), 9024(35b), 9234(6), 9277(6), 9346(2), 9491(29), 11015(20b), 11022(20b), 11049(20b), 11105(28b), 11108(28b), 11015(16), 11049(21), 11071(17), 11169(20a), 11170(17), 13318(29), 13346(35b), 13983(6), 14137(14a), 15015(29), 16718(14a), 16774(21), 17520(29), 17536(6), 17586(2), 17594(2), 17684(6), 18527(21), 18592(17), 18605(35a), 18652(17), 18676(17), 18680(38a), 18711 (28b), 18735(16), 19435(17), 19437(38b), 19466(28b), 19743(9), 20693(6), 20727(29), 21416(14a), 21419(29), 21508(6), 21805(35b), 21817(21), 22034(21), 22410(35b), 22440(21), 22804(28b), 22808(16), 24386(13), 24520(35a), 24522(18), 24616(39), 24650(29), 24686(19), 24736(19), 25857(11), 26378(14a), 26378 p.p.(29), 27037(13), 27214(29), 27417(29), 27141(19), 27214(29), 30048(13), 30129(18), 30479(21), 30564(14a), 30600(35b), 30747(14a), 30754(35b), 30764(35b), 30801(14a), 31136(13), 31276(13), 31303(37), 31563(29), 31618(29), 31698(6), 31713(21), 31769(5a), 31856(5a), 31596(35b), 31596 ½(29), 31700(14a), 31869(21), 32119(29), 32478(21), 32512(29), 32832(17), 32833(sp), 32834(17), 32840(17), 32850(30), 32856(30), 32864(17), 32867(9), 32881(24), 32952(39) 32964(7), 32971(9), 33009(17), 33011(9), 33012(17), 33013(20a), 33028(20a), 33030(17), 33034(38b), 33043 (20a), 33058(17), 33070(9), 33077(11), 33118(7), 33125(1), 33134(7), 33135(20a), 33151(33), 33157(11), 33158(11), 33169(11), 33195(10), 33196(11), 33249(11), 33251(10), 33252(24), 33256(11), 33261½(10), 33264(20a), 33277 (20a), 33277½(20a), 33193(20a), 33213(33), 33243(38a), 33252(7), 33253(20), 33257(1), 33284(17), 33286 (32), 33289(38a), 33308(11), 33315(21), 33328(28b), 33382(28a), 33391(24), 33397(1), 33420(24), 33441(24), 33466(28b), 33469(20a), 33474(28b), 33478(16), 33492(28a), 34149(33), 34154(11), 34155(11), 34157(28b); Cáceres 250(19); Cajal 1(21), 21(16), 125(5a), 136(16); Calcagnini s.n. BAB 13496(38b); Calderón 1122(19), 1069(39); Canevari s.n. BA 69511(20); Cano s.n.(38b), 112(38b), 330(17), 500(38b), 532(9), 1099(38b), 1227(35a),

4679(35a), 8312(34), 11872(19); Cantino 431(19); Caparelli 65(19); Cardenas 285(6), 312(21), 3740(6), 4912(21); Cardoso s.n. BA 23695(20a), s.n. BA 23731(33), s.n.(38a); Carette 5(28b), 62(16), 150(35a), 325(28b), 329(38b), 330(38b), 331(29), 332(29), 334(19), 335(17), 336(16), 337(11), 338(1), 3045(21), s.n.(33), s.n. BA 23670(39), s.n. BA 23677(19), s.n. LP 86591(33), s.n. LP8660(33), s.n. MERL 2564(6), s.n. MERL 3732(35a), 4032(33), s.n. SI 3328(35a), SI 3342(16), s.n. SI 3392(19), s.n. SI 3409(11), s.n. SI 27715(39); Castagno 34(28b); Castellanos s.n.(21), 80(28a), 160(24), 387(20a), 851(5a), 3541(9), 20160(17), 20288(20a), s.n. BA 6180(1), s.n. BA 6181(8), s.n. BA 6186(10), 7023(5a), s.n. BA 6175(7), s.n. BA 6176(33), s.n. BA 6177(33), s.n. BA 6178(20a), BA 7885(7), 7886(11), s.n. BA 7887(24), s.n. BA 7888(33), s.n. BA 14808(5a), s.n. BA 14811(29), s.n. BA 14813(19), BA 20163(21), s.n. BA 20166(29), BA 20211(38a), 20575(9), BA 23/709(21), s.n. BA 25/2939(21), s.n. BA 26/2272(35a), BA 27/204(21), BA 27/205(21), BA 27/210(21), s.n. BA 27/211(38a), s.n. BA 27/213(17), s.n. BA 27/848(5a), BA 27/889(21), s.n. BA 27/2027(19), s.n. BAB27/2032(39), 28/1195(20b), 28/1199(20b), 28/321(19), s.n. BA 28322(39), s.n. BA 28323(29), s.n. BA 30605(29), s.n. BA 30/738(5a), s.n. BA 36852(28a), s.n. BA 36853(20a), s.n. BA 36854(11), s.n. BA 36856(20a), s.n. BA 38860(19), s.n. BA 46962(5a), BA 49960(19), s.n. 46963(35b), BA 51175(14b), s.n. LIL 15202(29), s.n. LIL 15204(37), s.n. LIL 19297(13), LIL 20160(17), s.n. LIL 35318(29), LIL 35334(16), BA 36859(21); Castillo s.n.(33), 25(25); Castillón 300(29), 404(29), 436(5a), 959(19), 1081(29), 2073(5a), 2080(29), 2083(19), 2084(19), 2319(19), 2447(19), 2460(35b), 2501(5a), 2586(6), 3198(14a), 3199(19), 3200(29), 3229(5a), 3265(6), 3308(21), 3321(21), 3335(5a), 11984(19), 14213(19), 16393(19), s.n. LIL 13032(14a), LIL 13122(5a), LIL31588(5a), LIL 31587(5a), LIL 31594(5a), LIL 31595(5a), LIL 31597(5a), s.n. LIL 31603(6), s.n. LIL 32840(35b), s.n. LIL 32721(29); Castro s.n.(16); Catalano 12(5a), 14(21), BA 25/1515(21), BA 26/1720(21); Catedra de Geobotánica 5377(29), 5581(21); Ceballos 84(6); Cei s.n. MERL 26966(11), s.n. MERL 30189(33), s.n. MERL 30778(20), MERL 34970(16), s.n.(1); Cekalovic s.n.(33), 67(33); Cerrate 4859(19); Charpin 20799(21), 23185(6), 23111(21); Chicchi 43(LP 25564)(28b); Chong s.n.(31); Cialdella 194(14a), 251(21), 411(35b), 455(21); Citadini 1(11)52(20a); Claren 11400(2)(S, paratipo); Clemens s.n. Herb. Parques 8281; Clos 3547(21); Cocucci, A. 2702(29), 2267(19), 2282(28a), 2287(24), 2471(33), 2512(33); Collantes s.n.(27a); Comber 25(38b), 78(sp, 79)(17), 112(1) 133(17), 138(16), 189(30), 957(9), 1290(4); Cordo 522/78(19), s.n. 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26718(17),) 26762(39), 26811(1), 26835(28b), 26869(1), 26966(11), 26997(11), 27106(1), 27122(38a), 27133(11), 27197(35a), 27306(16), 27896(21), 28161(38b), 28478(32), 28490(38a), 28516(20b), 28533(38a), 28557(33), 28558(11), 28577(20a), 28632(8), 29204(35a), 29212(29), 30162(19), 30228(38b), 30304(20a), 30367(17), 30553(16), 30650(28b), 30680(16), 30683(38b), 30776(10), 30885(38b), 30969(28b), 30989(17), 31272(17), 31272(17), 31284(38b), 31294(38b), 31352(11), 31353(16), 31417(16), 31550(16), 31734(21), 31780(39), 32589(28b), 32675(28b), 32671(38b), 33110(29), 33087(29), 33877(11), 33907(19), 34510(28b), 34528(29), 34058(19), 34543(29), 34595(28b), 34910(38b), 36742(17), 36746(38b), 36792(28b), 37709(21), 40601(29), 40618(29), 40714(11), 14511(29), 14545(29), 14969(35a), 15169(28b), 15183(35a), 15227(37), 16561(37), 16729(29), 19039(29), 53633(28b), s.n. BA 62153(7); Merril King 9106(28b); Meyer 3136(29), 3369(19), 3486(21), 5186(21), 14621(19), 1970(2), 7300(20a), 7425(17), 7930(9), 7939(9), 8038(17), 8254(35b), 9586(11), 9594(11), 9687(11), 9721(17), 12119(35b), 13477(14a), 21561(19), 22727(14a), 22789(16), 23652(14a), 34408(35b), s.n. LIL 31407(29); Miccio Peralta 30(38b); Michl 261/80/19.1(37), 330/80/21.1(21); Miene 102/80/172(13); Miers 161(19); Milner s.n.(28a); Moldenke 19730(29); Molfino s.n. BAB 59666(21), s.n. BAB 59682(19), s.n. BAB 59683(28b); Molina 2438(11), 2348(11), 2672(10), 2677(20a), 2679(38b), 3498(19), 4650(20b), 4651(20b); Molina Massey 14(11), 51(38a); Montero 1283(25), 2313(7), 2660(11), 2729(7), 6017(25), 6643(25), 7076(25), 7078(25), 9627(25), 11328(25), 12485(25); Monticelli 18(35a), 11–39(35a), 1–50(35a), 44(17), s.n. SI 3358(17), 87(21), 73 p.p(21); Montiel s.n. Herb. Parques 2158(9); Moore 1281(11); Morello 1287(35b); Moreno 219(13), 291(13), 338(33), 608(33); Moreno y Tonini 335(9), s.n.(20a); Morrone 2133(14a), 2450(6), 2454(29), 2509(29), 2511(14a), 2593(2), 2595(6), 3131(29); Moseley s.n.(39); Moyano s.n.(39); Mühlenberg 31(20b); Múlgura 134(16), 211(21), 364(35a), 1267(21), 2927(14b); Muñoz 2495(33); Museo C. Nat. San Rafael 1852(21).

Nakamatser s.n.(20b); Naranjo 876(35a), 878(21), 897(19), 901(19), 904(29), 909(19), 911(18), 913(21); Navas 11(30), 27(1), 3806(19); Née 30149(6); Neuman 1038(35b), 1057(35b); Niemeyer s.n.(31), s.n.(39), 17(9), 376(9), 552(20a); Nichols & Eggero s.n.(6); Nicora 3672(39), 3680(9), 3684(39), 3776(39), 4263(28b), 4266(21), 4339(39), 4359(19), 4361(39), 4440(19), 3618(38b), 4445(29), 8179(21), 8261(21), 8413(38a), 8624(21), 8919(21), 8990(35b), 9183(14a), SI 14261(16); Niederlein 686(5a); Novara 8770(14a), 10424(21), 11037(21).

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Paci 8(19), 256(29), 813(21); Palacios 2037(19), 4417(19), 4420(19), 1542(21); Pardo 32(31), 60(5a); Parodi 2127(1), 4819(21), 4832(35a), 4834(21), 4843(21), 4849(35a), 7929(19), 8581(21), 12795(16); Partridge s.n. BA 30/180(13), s.n. BA 55118(13), s.n. 55124(13), s.n. BA 60731(37), s.n. BA 61933(13), s.n. BA 61935(21), s.n. BA 61937(19); Pearce s.n.(21); Pedersen 13248(21), 13256(9), 13317(21), 13332(35a), 14153(1), 14430(33), 14489(1); Pedreros 88(31); Peirano 32836(21), s.n.(19), s.n. LIL 32842(5a); Pemberton 12(33); Pennell 4162(19), 13187(34); Pennington 167; Pereyra 5881(29); Pérez Moreau R. A. 318(9), s.n. BAA 30/178(13), s.n. BA 30–179(16), s.n. BA 12704(19); s.n. BA 12706(19), s.n. BA 23354(28a), s.n. BA 45439(7), s.n. BA 45449(9), s.n. BA 48736(9), 55121(16), BA 55134(29), s.n. BA 61935(21), s.n. Ba 69967(33); Pérez Moreau R. L. 3113(28b), 3139(20a), 3152(17), 3192(17), 3219(30), 3221(1), 3244(9), 3263(28b), 3375(1), 3584(10), 3678(9), s.n.(1); Perrone s.n.(7), s.n. BA 5867(1), s.n. SI 20332(9), SI 20335(30), s.n., SI 20339(9), BA 58436(11), BA 58866(1), s.n. BA 61618(30), s.n. BA 61623(11), BA 70453(16), s.n. SI 20337(24), s.n. SI 20336(20a); Petersen & Hjerting 867(5a); Petersen 85(6), s.n. BA 45887(7), s.n. BA 45889(9); Pfister s.n.(27b), s.n.(33), 54(33), 12119(33); Philippi F., s.n. SGO 54801(5a); Philippi s.n.(5a), s.n. SGO 42479(21), SGO 42522(5a), s.n., pp.(Lectotipo SGO 54675)(15), s.n. SGO 54675 pp.(31)(SGO), SGO 54673(31), SGO 68374(31), s.n. año 1904(27a), s.n. SI 3447(13); Piccinini 1255(38b), 1300(9), 1332(20b), 1347(20b), 1532(38b), 1574(20b), 1580(20b), 1581(20b), 1652(20b), 2372(32), 3928(14b); Pichi Sermoli 7428(33); Pierrotti 7485(21)(K); Pisani 2715(33); Pisano 182(27b), 188(27b), 1396(39), 1399(39), 1792(5b), 1793(5b), 4180(33); Plotnik 3(38b), 27(17); Poeppig s.n.(36); Polsterflanzenflur & al. 4120(7); Pozner 128(21), 262(19), 266(20a); Pozzi 6438(20b); Prichard s/n(11); Prina 1190(21), 1200(20a), 1227(35a), 1228(21), 1283(38a), 1293(16), 1334(17), 1345(20b), 1351(20a), 1386(35a), 1397(21), 1454(38b), 1456(16), 1488(29), 1494(28b), 1543(11), 1566(24), 1577(16), 1625(29), 1781(21), 1784(29), 1790(21), 1879(1), 1868(28a), 1907(28b), 1935(20a), 2122(28b), 2251(1), 2390(38b), 2429(24); Propal 87(20), no. 154(20), 339(9); Pujalte 30(21), 34(13), 106(5a), 241(13).

Ragonese 27(21), 60(17), 124(7), 228(11), 351(28a), 352(21), 6060(14a), 7588(21), 7657(21), 8144(21), 9735(14b), s.n. BAB 69230(19); Rasp 78(21), 191(17); Reales 848(35a), 880(35b), 984(19), 991(21), 1088(19), 1065(19), 1115(21), 1148(21), 1206(35b), 1207(21), 1361(21), 1391(21), 1520(35b), 1525(21), 1609(14a), 1622(35b), 1641(21), 1649(35b), 1656(21), 1694(35b), 1776(14a), 1948(21), 1990(17), 2002(21), 2030(17), 2036(17), 2046(21), 2057(21), 2061(21); Reca 21(16), 37(21), 60(16), 69(16); Reed 170(19), s.n.(12); Reiche 13(5a), s.n. SGO 54677(29), SGO 54676(29); Renvoize 3396(19); Reyes s.n.(28b); Rhamer s.n. SGO 12172(15); Ricardi 51 s.n.(33), 76(34), 84(34), 120(34), 173(19), 191(19), 273(34), 297(31), 391(34), 434(2), 169(5b), 500(21), 753(26), 1368(34), 1404(21), 1550(27a), 1575(31), 1588(31), 1589(31), 1766(13), 2157(27a), 2441(25), 2487(27a), 2910(39), 3245(25), 3246(28a), 893(28a), 3378(6), 4272(27a), 4541(27b), 4659(21), 4674(5b), 1763(19), 4802(2), 4906(27b), 4635(27b), 4966(27b), 5513(21), 12815(27a), CONC 13886(28a), 25464(5a), 25595(2); Ringuelet 112(21); Rizzo s.n. BAB 78661(21); Rodrigo 3196(29); Rodríguez 284(19), 1218(29); Rodríguez, D. 1223(19), 1379(5a), 1984(19); Rodríguez Ríos 3170(31), 3198(31), 3595(31), 3646(31); Rohemeder 8(11), C 11(19), s.n. LIL 29388(5a); Roig 20(29), 365(1), 422(1), 610(19), 807(29), 2589(14b), 2914(29), 3163(18), 4648(19), 5286(19), 5400(35a), 5994(35a), 6279(38b), 6296(17), 6643(20a), 6648(1), 7269(38b), 8190(35a), 8909(38b), 9023(35a), 9255(33), 9810(1), 9812(38a), 9860(20b), 9863(20b),

10212(8), 10870(29), 10888(39), 10950(37), 10961(18), 12476(18), 13057(7), 13058(1), 13059(9) 13060(17), 13390(38b), 14060(38a), 14092(7), 14365(11), 14435(10), 14445(17), 14457(28b), 14692(33), 14537(33), 14580(10), 14669(11), 14683(38b), 14685(10), 14781(11), 16009(38b), 16015(7), 16023(28b), 16411(28a), 16032(38b), 23996 bis(10), 3219(30), 3221(1), 3219(30), 3221(1), s.n. TBPA-fIT 2397(8), s.n. TBPA-fIT 2452(8), s.n. TBPA-fIT 2453(1), s.n. TBPA-fIT 2478(8), s.n. TBPA-fIT 2841(20), s.n. TBPA-fIT 2867(8); Roivainen 2509(38b), 2660(33), s.n. BA 66335(33), s.n. BA 66363(38a), s.n. H 1061031(11), s.n. H 1061032(3); Romanczuk 961(33), 969(10), 1010(20a), 1055(1); Romanella 2(21); Roquero s.n. Herb. Parques 8932(33), s.n. Herb. Parques 8938(11); Rose 18834(6); Rossi 358(28a), 359(28a), 360(28a), 368(17), 369(20a), 370(24); Rossow 133(20a), 152(1), 166(20a), 196(9), 212(20b), 265(9), 540(9), 779(21), 942(28a), 1006(24), 1545(17), 1547(21), 1568(20b), 1657(20b), 1615(7), 1663(16), 1682(38b), 1723(35a), 1736(21), 1738(28b), 1739(17), 1743(21), 1785(28b), 2495(17), 2498(21), 2511(28b), 2528(7), 2686(28a), 2895(11), 3059(28b), 4491(28b), 4504(7), 4515(28b), 4550(11), 4585(1), 4586(28a); Roth s.n. LP 8615(17), s.n. LP 8375(38b); Rotman 345(35a), 374(37), 384(39), 395(19), 405(29), 408(19), 410(29), 414(16), 436(35a), 449(29); Rusby 2670(6); Ruthsatz 119(21), 136 15(14a), 227(2), 238(6), 273(2), s.n.(2), 314 21(14a), 367 b(35b), 373(29), 277(29), 6347(16), 6471(16), 6710(13), s.n. BAA 9991(29), BAA 9992(29), BAA 9993(29), BAA 9994(14a), BAA 9995(6), BAA 9996(2) BAA 9997(21), XXVI 14(14a), s.n.(6), s.n. BA 30/176(37).

Saénz 701(35a); Saile(37); Garay s.n.(37); Samler s.n.(36); Sánchez 145(21), 184(20b), 241(10), 244(11), 246(7), 252(9), 291(38a), 314(38a), 342(10), 344(33), 347(1), 381(20a), 384(11), 386(10), 486(10), 488(11), 520(20), 584(20b), 645(20a); Sanderman 3855(6)(K), 3891(34), 896(6), 3916(6); Sanso 121(28a), 135(28a); Sanzin 44(35a), 59(18), 120(39), 277(18), 554(21), 661(16), 733(29), s.n.(35a), 3102(35a), 3103(21), 3106(16), 3113(28b), s.n. MERL 2561(18); Sarasola 1099(19); Saravia Toledo 14725(14a), 14733(29), 14841(29), 14869(29), 14991(21), 14997(29), 15301(6), 15318(29); Sayago 410(14b), 519(35a), 600(35b), 616(21), 1507(21), 1937(19), 1962(35a), 2503(21), 2517(35a), 2731(19), 2819(21), 3245(17), 3316(7); Scala 4(17), 335(17), s.n. LP 8317(17), s.n. BA 23706(17); Schajovskoy 3295(28a), 33/IV(24), 35/IV(7), 36/IV(9), 43/IV(9), 051-iV(7), 54(20a),54-iV(30), 62/IV(7), 66-iV(7), 79/VII(10), 139/VII(9), 151/IV(9), 175(28a), s.n.(17), s.n.(38b), s.n(39), s.n. SI 25623(20a), s.n. SI 25624(39), s.n. SI 25625(1), s.n. SI 25628(38a), s.n. SI 25664(30), s.n. Herb. Parques 9315(28b); Schlegel 4827(19), 6205(25); Schinini 24530(21); Schikendantz 89(19); Schmitt 176(6); Schreiter 366(19), 631(14a), 1205(29), 1206(19), 2901(29), 4900(19), 4898(35b), 4899(29), 4901(29), 6083(21), 6094(5a), 6150(29), 7023(5a), 7034(19), 8713(19), 8829(14a), 10383(35b), 10047(29), s.n.(35a), 10048(35b), 10508(39), 10560(19), s.n. LIL 85703(19), s.n. LIL 86107(35b), LIL 86245(35b); Schub 5(27a); Schulz 1452(29), 2883(21), 6155(21), 6168(21), 6210(28b), 6216(6), 6241(21), 6608(19), 6668(19), 6675(19); Schwabe 510(2), 694(6), 708(2), 2079(21), 2097(17); Scolnik 176(6), 357(33), 365(33), 417(11), 423(10), 425(33), 428(11), 634(6); Seibert s.n. TBPA-fIT 2157, s.n. TBPA-fIT 2180(33); Seijo 1623(28b), 1713(28a), 2068(9), 2162(21), 2256(9); Selander 88-a–28(21); Semper s.n.(21), 28(35a), 119(21), 407(35a), 449(18), 574(29), 631(39), 4347(35a), s.n.(35b), MERL 9982(18), 10006(18); Senn 4305(17); Serra 29(13), 30(13), a 61(28b), 64(19); Sesberg 132(24); SGO 42507(25), 42480(24), 42483(12), 42526(27b), 54708(25), 54719(25), 54726(27b), 54802(12), 54682(24); Shepard 26(6); SI 2039(19), SI 3394(14b), SI 3408(1), 19178(14a); Silvestri 6470(11), 6471(20a), 6472(20a), 6473(38a), 6474(30), s.n.(8); Simpsom 8568(34); Skosttsberg 624(38b), s.n. BA 23725(11), s.n. CONC 18195(28a), s.n. SGO 58993(33), 11069(39), 11075(28b); Slanis 195(19), 219(14a); Sleumer 324(21), 381(16), 464(19), 515(14a), 547(38b), 589(24), 598(28a), 644(24), 664(19), 772(20), 897(20), 906(33), 1082(11), 1088(33), 1108(20a), 1109(20a), 1160(11), 1209(24), 1457(11), 1469(38a), 1916(5a), 2341(35b), 2364(19), 2677(35b), 2678(35b), 2679(21), 3228½(2), 3243(14a), 3316(2), 3319(2), 3345(35b), 3580(29), 6150(29), s.n.(8); Smith 4658(21); Solbrig 182(21); Solomon 4664(33), 12850(6); Soriano 550(1), 662(29), 1274(9), 1440(38b), 1500(1), 1870(20b), 1884(38b), 2003(11), 2014(3), 2038(7), 2044(3), 2060(7), 2065(8), 2101(11), 2117(11), 2136(20a), 2142(10), 2163(7), 2164(10), 2224(11), 2225(1), 2272(20a), 2433(38b), 2479(1), 2575(1), 2576(10), 2597(33), 2717(38b), 2728(20b), 2865(20a), 3211(10), 3284(20), 3344(20), 3385(11), 3388(33), 3402(20a), 3406(10), 3418(32), 3749(17), 3782(17), 3816(1), 3839(32), 3871(11), 3875(1), 3881(11), 3980(38b), 3985(10), 3989(10), 4006(20b), 4035(35a), 4048(17), 4057(19), 4072(28b), 4521(10), 4574(20a), 4700(11), 4701(11), 4733(10), 4764(11), 4781(10), 4781b(11), 4798(38b), 4814(11), 5037(38b), 5043(10), 5044(10), 5051(38a), 5095(33), 5096(20a), 5199(38b), 5264(10), 5265(10), 5266(20a), 5281(35a), 5282(20a), 5288(10), 5316(9), 5338(10), 5367(10), 5373(10), 5408(12), 5674(33); Sosa 32(16), 33(24); Soukup 43(6), s.n.(6); Sparre 5941(19); Spegazzini C. s.n.(19), s.n.(7), s.n.(33), 1122(11), 1134(8), 6476(35a), 6477(35a), 13069(11), s.n. BAB 11294(35a), BAB 22732(39), BAB 22733(39), BAB 22734(39), 23404(39), s.n. BAB 23152(35a), BAB 23451(35a), LP 10010(3), s.n.(14a), s.n. BAB 33722(19); Spegazzini P. L. s.n. BAB 29026(19); Spegazzini, R. 178(16), s.n. LP 10399, s.n. BAB 57952(35a), s.n. BAB 58190(18), s.n. BAB 61204(38b); Squo 88061(13); Stafford 333(19), 723(19), 1136(6); Steibel 2486(17), 2520(21), 3192(17), 4718(21), 5270(17), 5279(17); Stipanicic s.n. BA 44143(28b); Storni 29(39), s.n. BAB 69417(28b); Straw 2263(34); Stuckert 22289(20a); Stuessy 6811(11), 6897(11), 10255(28b), 10284(28a), 10355(21); Suárez 3(38b); Suero 1147(9).

Taylor 10649(27a), 11186(21), 11541(21), 11573(31), 15541(31); Teague s.n.(9)(K); Teillier s.n.(28b), 1017(27b), 2374(12), 3082(5b), 5309(28a), 5230(28a), 4583(25), 4584(25), 4585(13), 4666(85b), 5460(16), 5462(13); Tessleff 5272(20a), 5328(20b), 5399(38b), 5414(11), 5444(33), 6239(8); Thunger 107(33); Tome 149(39); Torres 53(21), 54(17), 97(17); Toursarkissian s.n.(9); Tovar 6779(19); Troiani 2778(21), 2796(35a), 3650(21), 15003(20b), 15043(20b), 15063(20a), 15068(20a), 15155(1), 15202(1), 15212(7), 15439(17), 15551(1), 15705(10), 15708(31), 15712(10), 15714(17); Troncoso s.n. SI 6062(19), s.n. SI 20498(21), s.n. SI 20513(17); Tutin 1374(21); Tweedie 311(33).

Ulibarri 747(21), 1480(21).

Valentín 42(20b), s.n. SI 3366(20b), s.n. SI 3445(33); Valla 33(35a) (BAA 17794), 39(21), 46(BAA 17807)(16), 55(20b), 65(28b), s.n. BAA 17796(17), s.n. BAB 17800(21), s.n. BAA 17826(28b); Vallerini 200(11), 234(9), 239(7), 542(20a), 638(17), 763(32), 900(20b), 907(38b), 986(20a), 1293(9), 1375(9), 1528(20a), 1533(17), 1552(20b), 2033(38b), 2040(20b), 2082(3), 2053(38a), 2076(11), 2115(11), 2124(10), 2126(20a), 2131(10), 2146(1),

2149(20a), 2167(1), 2172(33), 2324(28b), 2814(38b), 2825(39), 3387(17), 3388(sp, 3430)(7), 3439(17), 3478(16), 3492(28b), 3509(38b), 3520(38b), 3522(17), 3627(11), 3641(11), 3666(11), 3693(20b), 3772(20a), 3786(10), 3787(38b), 3803(1), 3805(35[a]), 3813(10); Vals 1006(19);Vargas 7927(34), 11348(6); Vattuone 199(21); Vázquez Avila 407(6); Venturi 4163(29), 4282(29), 4539(29), 4849(29), 5500(19), 6713(19), 6715(35b), 6718(19), 6720(39), 6941(19), 6943(5a), 6970(39), 8141(21), 8563(35b), 8597(35b), 8605(29); Vervoorst 3162(35b), 3212(5a), 3415(19), 3462(21), 3530(19), 4269(33), 5582(21), 5615(24), 5632(7), 5639(1), 5652(33), 5657(10), 5662(10), 5760(10), 5860(21), 5969(9), 7149(21), 7357(19), 7422(21), s.n. LIL 458752(19); Vidal 30(33), 39(11); Vilar s.n. BAB 44355(21); Villagran s.n.(39), 1358(31), 2328(34), 2430(19), 2474(19), 4063(19), 9072(21), 9107(5b), 9238(5b), 9529(31); Villamil 2257(1), 2259(10), 2281(11), 2282(11); Villanueva s.n. (31); Vogel 9523L(29); Volponi s.n.(21), 21(21), 24(21), 148(29), 319(21); von Rentzell s.n. SI 19233(39); Vuilleumier 300(6), 302(6), 387(21) 411(14a).

Wagenknecht s.n.(36), 112(39), 18421(36); Walter s.n.(9), s.n.(14a), s.n.(17); Weberbauer 5779(34), 6895(6), 7472(6); Weigend 97/771(34); Weisser 400(39); Weldt 126(39); Wердermann 231(13), 236(29), 488(39), 496(28b), 577(28a), 848 (27a), 1111(34), 1120(6); Werner 123(5b), 336(29), 444(29), 921(21); West 3909(27b); Wilczeck 37(1), 43(21), 45(21), 48(24), 49(38b), 51(35a), 53(35a) (paratipo G), isoparatipo (K, NY, S); Wingenroth 238(29), 319(39), 356(19); Worth 16692(39); Wulff A. 198(17), 200(21), 204(35a), 371(28a).

Yepes s.n. BA 18859(19).

Zardini 42(38b), 86(17), 148(29), 1931(29); Zöllner s.n.(31), 871(31), 998(31), 999(27a), 1374(13), 1486(13), 10964(28b), 3075(28b), 1537(28b), 5991(28b), 4091(19), 4397(31), 4402(6), 5183(25), 5533(26), 6482(33), 6528(28b), 7906(27b), 9875(26), 10019(31), 12533(27b); Zuloaga 5932(19), 5946(6), 6004(6), 6408(14a).

STAUFFERIA AND *PILGERINA*: TWO NEW ENDEMIC MONOTYPIC ARBORESCENT GENERA OF SANTALACEAE FROM MADAGASCAR[1]

Zachary S. Rogers,[2] Daniel L. Nickrent,[3] and Valéry Malécot[4]

ABSTRACT

Two new arborescent species of Santalaceae, both endemic to Madagascar, are described in the new monotypic genera, *Staufferia* Z. S. Rogers, Nickrent & Malécot and *Pilgerina* Z. S. Rogers, Nickrent & Malécot. Based on available molecular and morphological data, the new species are part of a clade formed with *Pyrularia* Michx. of Asia and North America, the Indo-Malesian genus *Scleropyrum* Arn., and the central and western African *Okoubaka* Pellegr. & Normand. *Staufferia* is distinguished morphologically from *Okoubaka* by the smaller inflorescences (4 to 10 vs. 50 to 100 flowers); smaller (1.8–2 × 1.1–1.6 cm vs. ca. 9 × 5 cm), obovoidal (vs. ellipsoidal) fruits; smaller (ca. 1.5 mm vs. 7–8 mm diam.), persistent perianth; thinner (0.5–1 mm vs. 15–20 mm thick), 5-sulcate (vs. smooth) exocarp; and thinner (ca. 0.5 mm vs. 3–4 mm thick), smooth (vs. deeply striate or alveolate) mesocarp. *Pilgerina* differs from *Scleropyrum* by the smaller inflorescences (8 to 23 vs. 60 to 100 flowers); pedicellate (vs. sessile) flowers; smaller (1.2–1.9 × 1.7–2.7 cm vs. ca. 3 × 2 cm), broadly transversely ellipsoidal to subspheroidal (vs. obovoidal to pyriform) fruits; and thinner (ca. 0.5 mm vs. 1.5–3 mm thick), smooth or finely striate (vs. deeply striate or alveolate) mesocarp. Both species are illustrated and assigned an IUCN preliminary conservation status of Least Concern (LC).

Key words: IUCN Red List, molecular phylogeny, *Pilgerina*, Santalaceae, Santalales, *Staufferia*, taxonomy.

Cavaco and Keraudren (1955), in their treatment of Santalaceae for the *Flore de Madagascar et des Comores*, recognized the shrubby genus *Thesium* L. as the only member of the family occurring in Madagascar. Two years later, Capuron (1957) noted that Olacaceae were represented on the island by five genera, *Anacolosa* Blume, *Olax* L., *Phanerodiscus* Cavaco, *Ximenia* L., and by a fifth undescribed genus only known from a single collection (*Service Forestier 987*), which differed markedly from these named taxa in having five alternisepalous longitudinal furrows on the external surface of the fruit, thereby giving the false impression of a dehiscent 5-locular capsule. In an ineffectively published technical report, Capuron (1968: 39–40) recognized the material as an unplaced arborescent taxon of Santalaceae providing the provisional name "Santalacée A." Within that same document, he attributed three other collections (*H. Humbert 28660*, *Service Forestier 22622*, *23390*) to a second taxon, recognized as "Santalacée B" (Capuron, 1968: 40–41). At that time, both taxa were only known from fruiting material, but specimens still showed well-preserved, persistent floral organs remaining at the apex of the fruit. Capuron, so sufficiently confident that the two santalaceous taxa were unknown to science, provided provisional descriptions and illustrations for both in his report. Since that report, however, neither taxon has received much attention, but over the course of the past 15 years, additional flowering and fruiting specimens have been collected, as well as leaf material preserved in silica gel, that allow us to reexamine Capuron's work and to specifically address the phylogenetic position of his "Santalacée A" and "Santalacée B" using molecular and morphological means.

MATERIALS AND METHODS

PHYLOGENETIC ANALYSIS

The specimens used for DNA extraction are listed in Table 1. Leaf samples were taken from either silica

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Table 1. Voucher information and GenBank accession numbers for the 11 taxa from the Santalaceae used in the phylogenetic analyses. The DNA accession numbers refer to the collection by D. L. Nickrent archived at SIUC.

Taxon	Voucher	Country	DNA accession number	GenBank accession numbers			
				SSU	*rbcL*	*matK*	*accD*
Acanthosyris asipapote M. Nee	*M. Nee & I. Vargas C. 45009* (NY)	Bolivia	4051	DQ329163	DQ329171	DQ329182	DQ329193
Acanthosyris falcata Griseb.	*M. Nee 46690* (NY)	Bolivia	4053	DQ329164	DQ329172	DQ329183	DQ329194
Buckleya distichophylla (Nutt.) Torr.	*L. Musselman s.n.* (SIU)	U.S.A.	2735	X16598	DQ329180	DQ329191	DQ329202
Cervantesia tomentosa Ruíz & Pav.	*L. Dorr & L. Barnett 6941* (MO)	Bolivia	4273	DQ329165	DQ329173	DQ329184	DQ329195
Comandra umbellata (L.) Nutt.	*D. Nickrent 2739* (SIU)	U.S.A.	2739	DQ329170	DQ329181	DQ329192	DQ329202
Jodina rhombifolia (Hook. & Arn.) Reissek	*M. Nee 46673* (NY)	Bolivia	4052	DQ329166	DQ329174	DQ329185	DQ329196
Okoubaka aubrevillei Pellegr. & Normand	*M. Cheek 6007* (K)	Cameroon	4173	—	DQ329175	DQ329186	DQ329197
Pilgerina madagascariensis Z. S. Rogers, Nickrent & Malécot	*R. Rabevohitra et al. 4485* (MO)	Madagascar	4954	DQ329169	DQ329178	DQ329189	DQ329200
Pyrularia pubera Michx.	*L. Musselman s.n.* (SIU)	U.S.A.	2737	L24415	DQ329179	DQ329190	DQ329201
Scleropyrum pentandrum (Dennst.) Mabb.	*S. Suddee et al. 1007* (TCD)	Thailand	4347	DQ329167	DQ329176	DQ329187	DQ329198
Staufferia capuronii Z. S. Rogers, Nickrent & Malécot	*R. Randrianaivo et al. 825* (MO)	Madagascar	4956	DQ329168	DQ329177	DQ329188	DQ329199

gel–dried leaves or fresh material. *Comandra umbellata* (L.) Nutt. and *Buckleya distichophylla* (Nutt.) Torr. were used as outgroups. A modified 2× cetyltrimethylammonium bromide (CTAB) method was employed for all genomic DNA extractions (Nickrent, 1994). The polymerase chain reaction (PCR) mixture (final concentration) contained 1× Promega buffer (5 mM KCl, 1 mM TrisHCl), 1.5 μM $MgCl_2$, 50 μM dNTPs, 0.4 μM forward and reverse primers, 1 Unit Taq polymerase, 30–50 ng genomic DNA. Nuclear small-subunit (SSU) rDNA and three chloroplast genes (*rbcL*, *matK*, and *accD*) were amplified. PCR amplifications for SSU rDNA and *rbcL* generally followed the conditions outlined in Nickrent (1994). For amplification of *matK* and *accD*, a step-up procedure modified from Palumbi (1996) was used: 94°C for 5 min. followed by 5 cycles at 94°C for 30 sec., 46°C for 30 sec., 65°C for 90 sec. followed by 25 cycles at 94°C for 30 sec., 48°C for 30 sec., and 68°C for 90 sec. The following primers were typically used for PCR amplifications and sequencing (all 5′ to 3′): for SSU rDNA, 12f (TCC TGC GAG TAS TCA TAT GC), 1131r (CAA TTC CTT TAA GTT TCA GCC), and 1769r (GAC GTA CGG AAA GCT TGT T); for *rbcL*, 1f (ATG TCA CCA CAA ACA GAR AC), 635f (GC GTT GGA GAG ACC GTT TC), and 3r (TAG TAA AAG ATT GGC CCC AC); for *matK*, 78f (CAG GAC TAT ATT TAT GCA CT) and 1420r (TCG AAG TAT ATA CTT TAT TCG); and for *accD*, 1f (TCT ATG GAA AGA TGG YGG TT), 1Bf (ATG GAA AAA TGG YGG TTY AA), and 1300r (TCY TCA ATT ACT CTT TTA CC). Sequencing was conducted using automated methods (ABI Prism 377 automated DNA sequencer; Applied Biosystems, Foster City, California) according to manufacturer's protocols. Sequences were deposited with GenBank under the accession numbers given in Table 1.

The above ingroup and outgroup sequences were aligned manually using SeAl version 2.0 (Rambaut, 2004), and, for the three protein-coding genes, alignment was guided by assumed amino acid composition. The multigene alignment is available on the second author's web site: http://www.parasiticplants.siu.edu/Alignments/Alignments.html. Analyses of the separate gene partitions showed that all were generally congruent, hence the four genes were concatenated, thereby producing a matrix of 11 taxa by 5694 sites. Gaps were treated as missing data. The data matrix was analyzed using maximum parsimony (MP) as implemented by PAUP* 4.0b10 (Swofford, 2002). A branch-and-bound MP search was performed using 100 random addition sequence replicates with tree-bisection-reconnection (TBR) branch-swapping, holding 10 trees at each addition step, with all sites equally weighted. Nodal support was estimated using equal-weights MP

Table 2. Gene diversity statistics for all data partitions for the 11 taxa of Santalaceae. The nuclear SSU rDNA sequence was not obtained for *Okoubaka aubrevillei*. CI– = Consistency index minus uninformative sites; cp = chloroplast; HI– = homoplasy index minus uninformative sites; RI = retention index; RC = rescaled consistency index.

Data partition	Sites	No. trees	Tree length	Parsimony informative sites	CI–	HI–	RI	RC
Nuclear SSU rDNA	1813	4	160	39	0.7414	0.2586	0.7826	0.7092
Chloroplast *rbcL*	1421	2	176	60	0.7204	0.2796	0.8129	0.6929
Chloroplast *matK*	1180	1	377	121	0.7606	0.2394	0.8289	0.7300
Chloroplast *accD*	1280	1	242	83	0.8598	0.1402	0.9148	0.8581
Combined cp genes	3881	1	801	264	0.7665	0.2335	0.8408	0.7443
Combined four genes	5694	1	961	302	0.7633	0.2367	0.8346	0.7417

bootstrap analysis (heuristic search using 1000 random sequence addition replicates).

TAXONOMY

Herbarium specimens were examined from G, MO, NEU, P, TAN, and TEF. Additional material of *Okoubaka* Pellegr. & Normand and *Scleropyrum* Arn. was studied from several additional herbaria (B, BR, K, L, LAE). Observations and measurements for *Okoubaka* were predominately based on an examination of dried specimens of both members of the genus, *O. aubrevillei* Pellegr. & Normand and *O. michelsonii* J. Léonard & Troupin. Our morphological observations for the poorly known *Okoubaka* were supplemented by additional flowering and fruiting information taken from several pertinent literature sources (Louis & Léonard, 1948; Léonard & Troupin, 1950; Villiers, 1973a, b; Hallé, 1987). For *Scleropyrum*, the measurements and observations provided below are based mostly on herbarium material of the two Asiatic species, *S. pentandrum* (Dennst.) Mabb. and *S. maingayi* Hook. f., which are the most morphologically similar to the Malagasy taxa. Two other taxa, *S. aurantiacum* (K. Schum. & Lauterb.) Pilg. and *S. leptostachyum* Pilg., both from New Guinea, probably do not belong to *Scleropyrum* (Malécot, pers. obs.), and thus were not included here. Additional morphological information regarding the flowers and fruits of *Scleropyrum* was taken from the most recent, comprehensive literature (Macklin & Parnell, 2000, 2002; Nianhe & Gilbert, 2003).

The botanical illustrations and floral measurements of the Malagasy species were made from rehydrated material. Complete collection data for cited exsiccatae, including photos of types and other representative specimens, are posted on the Tropicos database at http://www.tropicos.org. Coordinates and elevations of collecting localities were assigned post-facto, when necessary, using the "Gazetteer to Malagasy Botanical Collecting Localities" (Schatz & Lescot, 2007; http://www.mobot.org/MOBOT/Research/madagascar/gazetteer/), and are enclosed by square brackets in the text. The distribution map was created using ArcGIS 9, and species distributions are superimposed over the five simplified bioclimatic zones of Madagascar (Schatz, 2000, following Cornet, 1974). Conservation status for each species is provisionally assigned based on the *IUCN Red List Categories and Criteria Version 3.1* (IUCN, 2001).

RESULTS

PHYLOGENETIC ANALYSIS

Gene diversity statistics from MP analyses of each of the individual gene partitions are shown in Table 2. The lowest number of parsimony informative sites (39) was obtained from the SSU rDNA partition and the highest (121) from *matK*. The highest consistency index (CI–, consistency index minus the uninformative sites) was from the *accD* partition (86%) and the lowest came from *rbcL* (72%). Only *matK* and *accD* resulted in one most parsimonious tree, whereas SSU rDNA produced four, and *rbcL* yielded two equal-length trees. Combining all three chloroplast gene partitions resulted in one tree that was identical in topology to the one obtained with the four-gene data set that included nuclear SSU rDNA (Fig. 1). The tree expands the well-supported "clade b" previously described in Nickrent and Malécot (2001: 71) by the addition of *Cervantesia* Ruíz & Pav., *Scleropyrum*, Capuron's Santalacée A (= *Staufferia* Z. S. Rogers, Nickrent & Malécot), and Santalacée B (= *Pilgerina* Z. S. Rogers, Nickrent & Malécot). The nine ingroup taxa form two well-supported clades (Fig. 1, 100% bootstrap support [BS]), the first (the *Cervantesia* clade) composed entirely of the South American genera *Acanthosyris* (Eichler) Griseb., *Cervantesia*, and *Jodina* Hook. & Arn. ex Meisn., and the second (the *Pyrularia* clade) of the remaining genera, namely *Pyrularia* Michx., *Pilgerina*, *Staufferia*, *Scleropyrum*, and *Okoubaka*. All members of the latter clade are Old World taxa with the exception of the basal genus

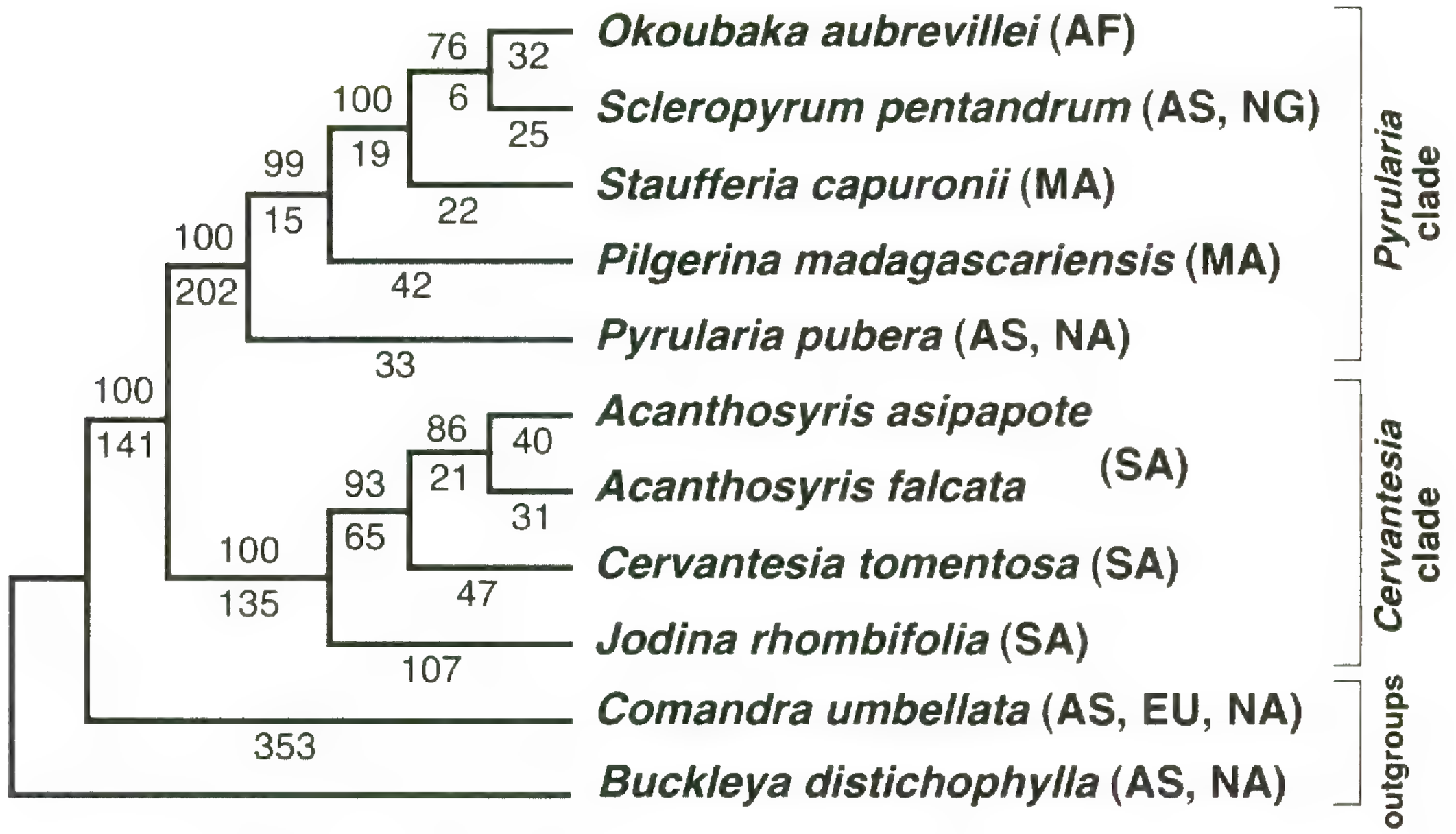

Figure 1. The single MP tree obtained from the analysis of a combined SSU rDNA, *rbcL*, *matK*, *accD* data set for the 11 species and 10 genera of Santalaceae used in this study. BS given above branches and branch length (number of steps) below. See Table 2 for tree statistics. The geographic distribution for each genus is enclosed in parentheses to the right of the constituent species. AF = Africa, AS = Asia, EU = Europe, MA = Madagascar, NA = North America, NG = New Guinea, SA = South America.

Pyrularia, present in eastern Asia as well as eastern North America. *Okoubaka* appears as sister to *Scleropyrum*, although BS is only moderate (Fig. 1, 76% BS). *Staufferia* is sister to the *Okoubaka–Scleropyrum* clade (100% BS) with *Pilgerina* sister to those three genera (99% BS).

Taxonomy

Staufferia Z. S. Rogers, Nickrent & Malécot, gen. nov. TYPE: *Staufferia capuronii* Z. S. Rogers, Nickrent & Malécot.

Hoc genus ab *Okoubaka* Pellegr. & Normand inflorescentia staminata minore (4- ad 10- vs. 50- ad 100-flora), fructu minore obovoideo (vs. ellipsoideo), perianthio in fructu persistente minore, pyrena minore (1.1–1.6 vs. plus quam 3 cm in diametro) obovoidea (vs. ellipsoidea), exocarpio 0.5–1 (vs. 15–20) mm crasso longitudinaliter 5-sulcato (vs. laevi) atque mesocarpio laevi (vs. profunde striato vel alveolato) ca. 0.5 (vs. 3–4) mm crasso differt.

Dioecious shrubs or trees; branches estipulate. Leaves alternate, distichous, margins entire; venation brochidodromous. Inflorescences axillary; staminate inflorescences thyrsoid, 4- to 10-flowered, bracteate; pistillate inflorescences not seen. Flowers unisexual, obovoidal (in bud), 5-merous, epigynous, actinomorphic, sessile; calyx absent; petals 5, free, valvate; staminate flowers with 5 oppositipetalous stamens (adnate near base of petal lobe and below the adjacent disk), introrse, dorsibasifixed; anthers tetrasporangiate (two pairs of divaricate locules), each loculus of a theca opening by a common, longitudinal slit; nectary disk star-shaped, fleshy, located between stamen and pistillode style; disk lobes 5, triangular, entire, apex rounded, each lobe alternating with a filament; pistillode style ± orbicular in transverse section; pistillate flowers not seen (based on persistent remains at the apex of the fruits): staminodes 5, minute, hidden under the apical lobes of fruit exocarp; ovary inferior, 1-locular; placental column straight; ovules 1 to 3, apical, pendulous; style very short cylindric or absent; stigma subsessile, with 3 to 5 lobes. Fruits drupaceous, single-seeded, obovoidal, with persistent floral parts at apex; persistent perianth ca. 1.5 mm diam.; exocarp fleshy, 0.5–1 mm thick, with 5 fused indehiscent segments, each segment alternating with a longitudinal densely pubescent furrow; pyrene (i.e., seed plus mesocarp) obovoidal, 1.1–1.6 cm diam., with a bony, smooth, ca. 0.5 mm thick mesocarp; endocarp papyraceous, very thin. Seeds globose; endosperm copious.

Etymology. The genus is named in honor of Hans Ulrich Stauffer (1929–1965), whose 10 "*Santalales-Studien*" publications (e.g., Stauffer, 1957, 1961, 1969) made major contributions to our understanding of Santalaceae. His unexpected death in 1965 prevented him from summarizing his morphological observations and taxonomic conclusions that would have eventually resulted in a complete monograph of

the family. One of his most astute observations was that some members of the heterogeneous tribe Osyrideae, with its two superimposed loculae per theca, should be segregated, as was done posthumously (Stauffer, 1969) as Amphorogyneae. It is significant that early in his career Stauffer (1957) recognized the affinity of *Okoubaka* (a large East African tree that was nebulously placed in either Octoknemaceae or Olacaceae) with *Scleropyrum* and *Pyrularia*, and that these genera plus *Staufferia* have now been shown to be closely related using molecular data. The description of *Staufferia* marks the first time a genus has been named in Stauffer's honor.

Staufferia capuronii Z. S. Rogers, Nickrent & Malécot, sp. nov. TYPE: Madagascar. Antsiranana Prov.: Analabe forest, Fivondronana Vohémar, Firaisana Nosibe, Fokotany Anjiabe, forêt littoral sur sable d'Anaborano, près du Lac Sahaka, 13°04′42″S, 49°54′13″E, 25 m, 2 Nov. 2002 (♂ fl.), *J. Rabenantoandro, R. Rabevohitra, G. McPherson, H. Ranarivelo & M. Sola 1100* (holotype, MO!; isotypes, G!, INH!, K!, P!, TEF!). Figure 2.

Dioecious shrubs or trees to 20 m tall; buds and young vegetative organs densely to moderately sericeous, sparsely pubescent or glabrous on mature branches; trichomes 0.3–0.5 mm, simple, unbranched, adpressed or subadpressed; branches ± zig-zag; bark longitudinally striate, cracked and exfoliating when dry, sometimes lenticellate. Leaves 2–7 × 1–3 cm, length:width ratio (2–)2.5–3:1; leaf blades elliptic or ovate, adaxially glabrous, abaxially moderately pubescent to glabrescent, chartaceous, apex acuminate or acute, acumen 3–8 mm when present, margins revolute, base short attenuate; midrib depressed adaxially, prominently raised abaxially, more pubescent than the blade; venation usually more strongly raised abaxially; secondary veins 4 to 6 pairs per side; fine venation very loosely reticulate, rarely obvious adaxially; petioles 2–5 mm, pubescent to less often glabrous, caniculate adaxially, rounded abaxially, articulate at base. Staminate inflorescences 5–7 mm, 4- to 10-flowered (with 2 or 3 subsessile flowers at the tip of each inflorescence axis), 3- to 5-branched, located near the base of young shoot with the main axis subtended by 1 or 2 scales or rarely by a leafy bract or developed leaf (at early stage, a young shoot with several inflorescences may be interpreted as a long inflorescence, but young leaves are located near the apex of the axis); inflorescence axes ca. 0.3 mm diam., densely pubescent; primary inflorescence axis 1.5–2.5 mm; bracts linear, 0.8–1 × 0.2–0.3 mm, densely pubescent abaxially, otherwise glabrous. Flowers sessile; staminate flowers: 1–1.5 mm, 1–2 mm diam., obovoidal (in bud), yellow or yellow-green, externally densely to moderately sericeous, trichomes in a pattern resembling a calyx; calyx absent; petals ovate, 0.8–1 × 0.7–1 mm, papilliate near apex, along upper 1/3 of margin, and along base adaxially, puberulent behind filament adaxially, those trichomes 0.1–0.2 mm, matted and wavy (trichomes weakly attached to the abaxial surface of the filament near the anther connective), moderately to sparsely pubescent abaxially, those trichomes 0.1–0.3 mm, straight; filaments 0.25–0.3 mm, 0.15–0.2 mm wide (at base), glabrous; anthers ca. 0.3 × 0.4 mm, glabrous; nectary disk ca. 1 mm diam., fleshy, glabrous; disk lobe 0.3–0.4 × 0.4–0.5 mm; pistillode style ca. 0.3 mm diam., papilliate, sometimes with a few distinct lobes; pistillate flowers not seen. Fruits drupaceous, obovoidal, 1.8–2 cm, (1.1–)1.4–1.6 cm diam., light yellow, 1(2) developing per infructescence; fruiting pedicels 2.5–4(–6) mm, 0.8–2 mm diam., articulate and thicker where meeting the fruit; exocarp fleshy, 0.5–1 mm thick; mesocarp bony, ca. 0.5 mm thick; endocarp papyraceous, ca. 0.1 mm thick. Seeds globose, 1.1–1.3 cm diam.; embryo 5–6 mm, incurved.

Distribution and phenology. *Staufferia capuronii* is widespread but patchily distributed in the eastern half of Madagascar from 25–1827 m elevation (Fig. 3). Populations are distributed from the humid wet forest of the Montagne d'Ambre in the far north of the island to the sandy littoral forest of Mandena in the southeast (near Fort Dauphin, Toliara province). The species has been recorded in flower in October, November, February, and May. Fruiting collections were made in January, February, and September.

Conservation status. The species occurs inside two protected areas (Mandena, Montagne d'Ambre). Some of the population located near Zahamena National Park may actually fall within park boundaries. The extent of occurrence (EOO) of the species is 81,000 km^2, and the area of occupancy (AOO) is 60,000 km^2 based on a 100-km^2 grid cell size. The species is assigned a provisional IUCN conservation status of Least Concern (LC) (IUCN, 2001).

Etymology. The epithet was chosen to honor René Capuron (1921–1971), as he was the first to collect this species and bring attention to this novel component of the Malagasy flora.

Discussion. Several examined collections exhibit notable morphological differences from plants made at the type locality: *M. Callmander et al. 317*, a fruiting collection made about 140 km west of the type locality and at higher (ca. 500 m) elevation, has

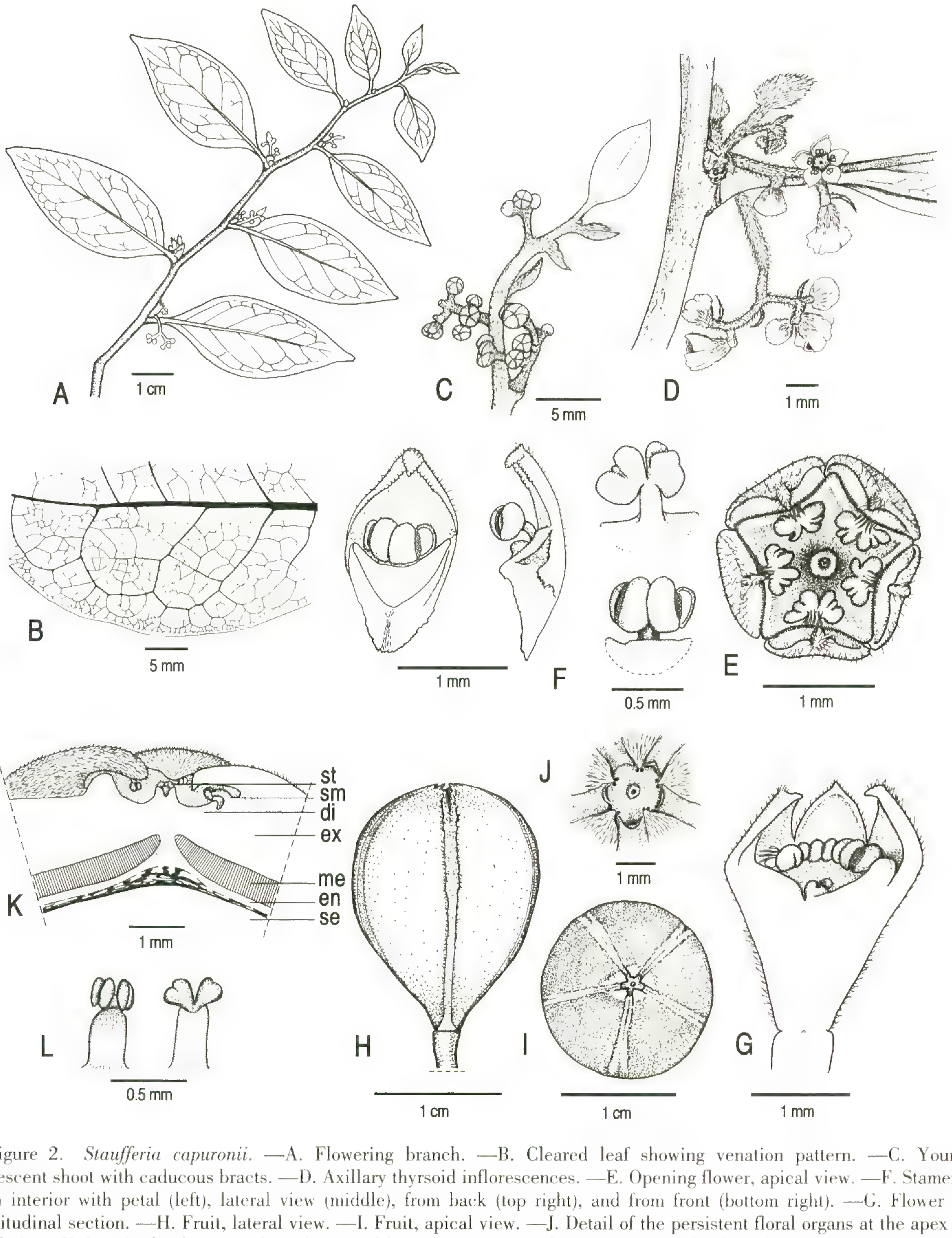

Figure 2. *Staufferia capuronii.* —A. Flowering branch. —B. Cleared leaf showing venation pattern. —C. Young pubescent shoot with caducous bracts. —D. Axillary thyrsoid inflorescences. —E. Opening flower, apical view. —F. Stamens from interior with petal (left), lateral view (middle), from back (top right), and from front (bottom right). —G. Flower in longitudinal section. —H. Fruit, lateral view. —I. Fruit, apical view. —J. Detail of the persistent floral organs at the apex of the fruit. —K. Longitudinal section through apex of fruit; st = stigma/style, sm = staminode, di = disk, ex = exocarp, me = mesocarp, en = degenerated endocarp, and se = seed endosperm. —L. Staminode from below apical lobe of fruit, showing interior (left) and exterior (right) surfaces. All drawn from *Service Forestier (Capuron) 987* (P).

glabrescent buds and shoots and a nearly glabrous fruit surface (Fig. 4A, B); *L. Nusbaumer et al. 1436*, collected ca. 25 km southwest of the type locality and made on lateritic soil rather than sand, has more densely pubescent buds, shoots, and infructescence axes, and a denser indument over the surface of the immature fruits (Fig. 4C); *J. Rabenantoandro et al. 161*, an immature fruiting collection made ca. 400 km south of the other localities, has consistently smaller and more pubescent leaves (but still of the same shape). The leaf and pubescence variation we have observed in these specimens may be related to environmental factors, whereas the fruit differences could be due to different stages of maturity. At

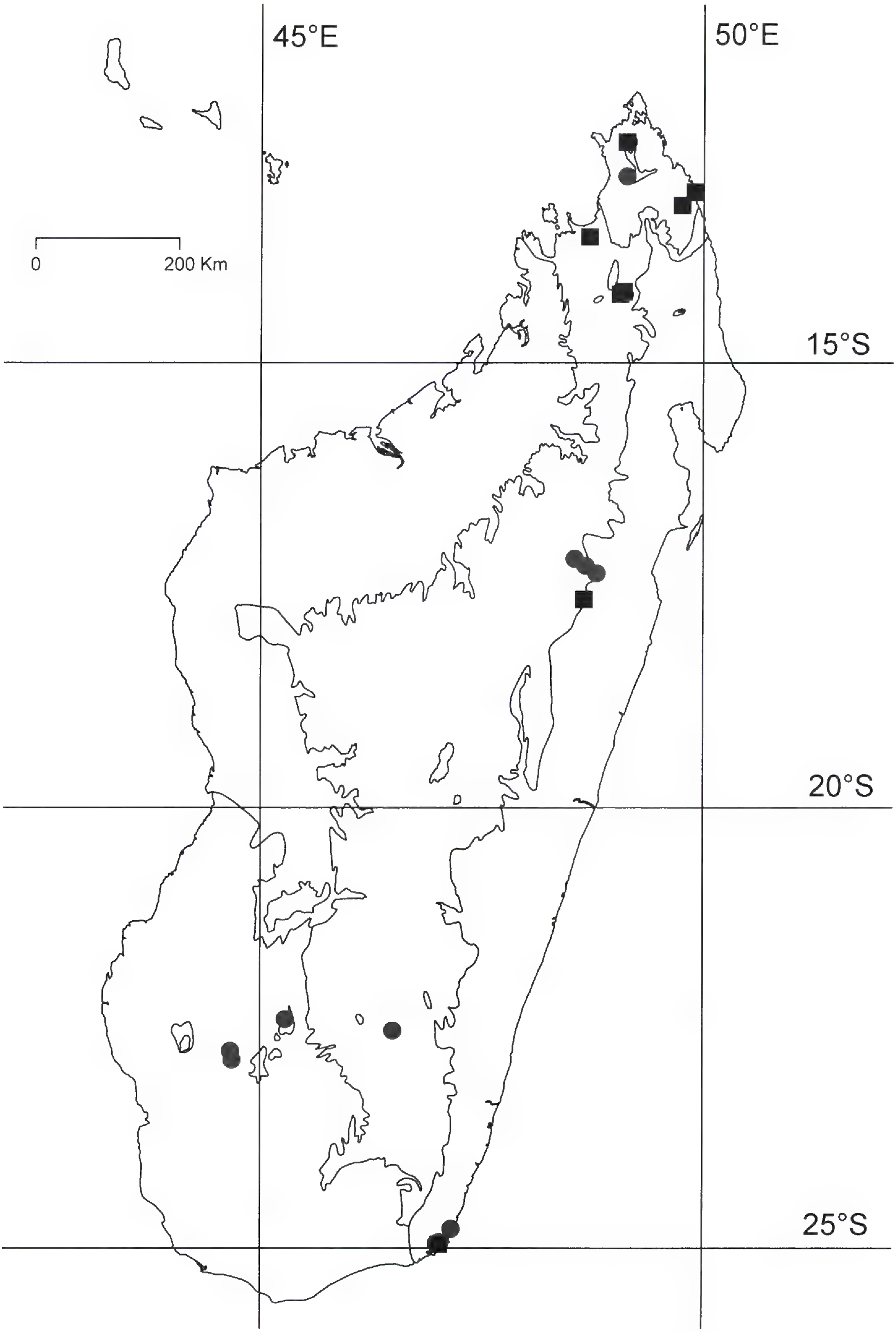

Figure 3. Distribution of *Pilgerina madagascariensis* (shaded circle) and *Staufferia capuronii* (square) on Madagascar.

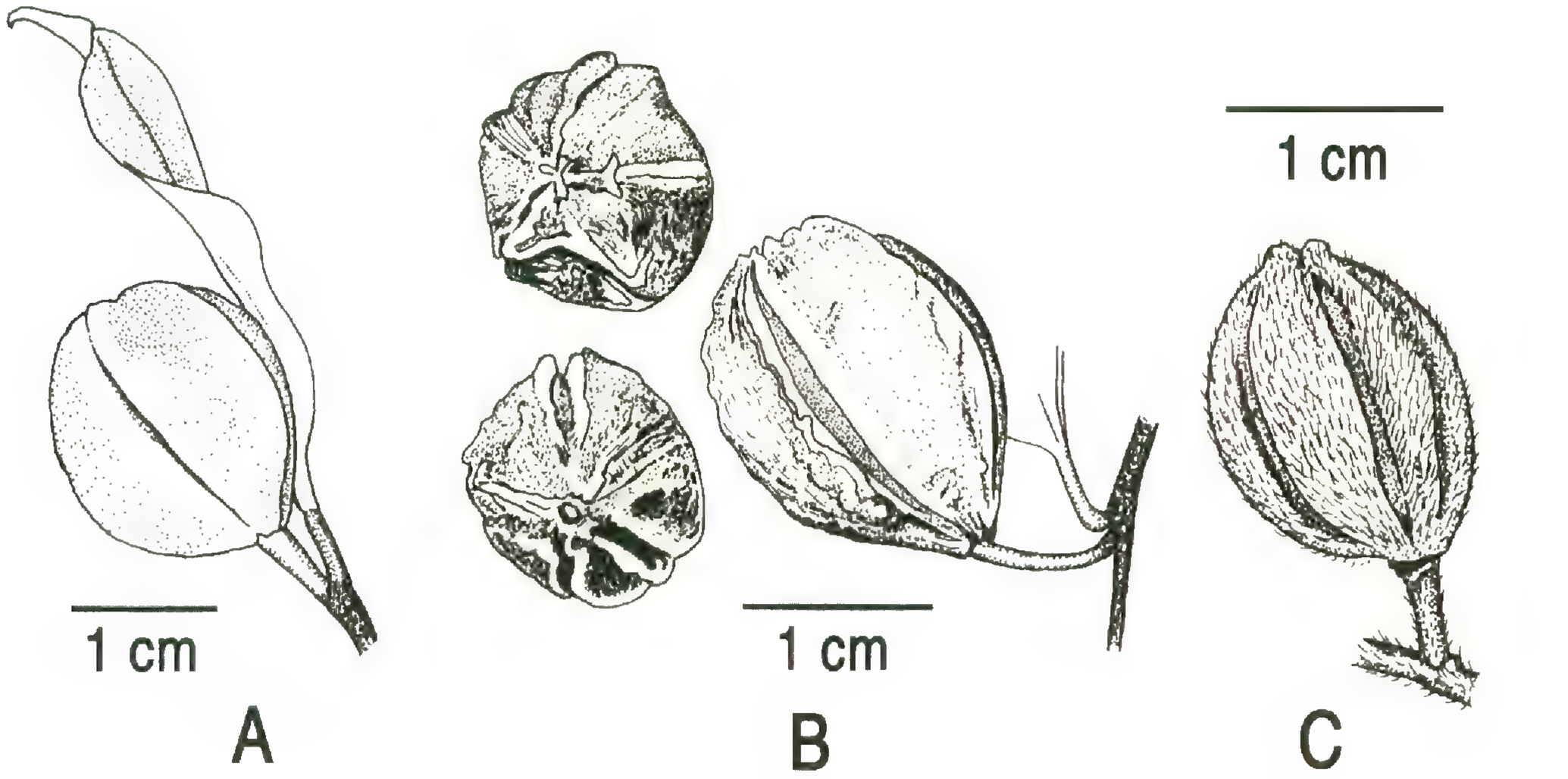

Figure 4. Fruit variation in *Staufferia capuronii*. —A. From a fresh fruit (*M. Callmander et al. 317*, photo). —B. From the same collection as A but dry (right), apical portion (upper left), basal portion (lower left). —C. Immature fruit from a dry specimen (*L. Nusbaumer et al. 1436*, photo).

present, sufficient material is not available to adequately address whether these three collections are sufficiently distinct to warrant taxonomic recognition.

Paratypes. MADAGASCAR. **Antsiranana Prov.:** Ambilobe, Commune Rurale Beramanja, chaine Galoka, Mont Galoka, Fokontany Anketrabe-Belinta, 13°35′07″S, 48°42′57″E, *M. Callmander, S. Buerki & C. Manjaribe 317* (G, MO[2], P, TEF); Analabe forest, Fivondronana Vohémar, Firaisana Nosibe, Fokotany Anjiabe, 13°04′45″S, 49°54′17″E, *R. Rabevohitra, J. Rabenantoandro & R. Razakamalala 4485* (MO, P, TEF), *R. Razakamalala, D. Rabehevitra & S. Mathieu 1254* (BR, MO, P, TEF); Anivorano Nord, Marotaolana, Analamahitsy, 10 km au Sud d'Anivorano Nord, au bord de la rte. nationale no. 6, 12°50′10″S, 49°13′42″E, *F. Ratoroson, J. Razafitsalama & R. Guittou 877* (TAN), *Z. Rogers, F. Ratovoson, R. Ranaivojaona, C. Davidson & S. Christoph 1166* (MO, P, TAN); Parc National Montagne d'Ambre, ca. 4 km WSW of Gite Etape, [12°31′S, 49°08′E], *S. Malcomber & S. Rapanarivo 1201* (P); Sous-prefecture Vohémar, Commune Rurale de Daraina, Massif de Bobankora, partie Nord, 13°13′39″S, 49°45′20″E, *L. Nusbaumer, S. Wohlhauser & P. Ranirison 1436* (G, MO, P, TEF). **Mahajanga Prov.:** Ambohimirahavavy, Massif de l'Ambohimirahavavy, rebord S du plateau de Marofamamo, Bealanana, [14°12′S, 49°06′E], *Service Forestier (Capuron) 987* (MO, P[2], TEF); Bemafo, Versant Nord Ouest du campement Bemafo, 14°13′48″S, 49°03′32″E, *C. Rakotovao, S. Buerki, J. Vazaha & Torize 2349* (G, K, MO[2]). **Toamasina Prov.:** Vohitsingitry, à 10 km SE du village d'Andranomalaza Sud, juste au dehors du parc national de Zahamena, à 800 m de la limite, 17°39′43″S, 48°39′28″E, *J. Rabenantoandro, S. Rakotonandrasana & I. Rak 161* (G, K, MO[2], P, TEF). **Toliara Prov.:** Forêt littorale à Mandena RF, Ampasi-Nahampoana, Fort-Dauphin, 24°57′26″S, 47°01′34″E, *F. Ratovoson, F. Randriatafika, J. Rabenantoandro & E. Ramisy 113* (MO, P, TEF).

Pilgerina Z. S. Rogers, Nickrent & Malécot, gen. nov. TYPE: *Pilgerina madagascariensis* Z. S. Rogers, Nickrent & Malécot.

Hoc genus a *Scleropyro* Arn. inflorescentia minore (8- ad 23- vs. 60- ad 100-flora), floribus pedicellatis, fructu minore (1.2–1.9 × 1.7–2.7 cm vs. ca. 3 × 2 cm) lato transverse ellipsoideo usque subsphaeroideo (vs. obovoideo usque pyriformi), pyrena lato transverse ellipsoidea usque subsphaeroidea (vs. obovoidea usque subsphaeroidea) atque mesocarpio laevi vel minute striato (vs. profunde striato vel alveolato) ca. 0.5 (vs. 1.5–3) mm crasso differt.

Shrubs or trees; branches estipulate, glabrous. Leaves alternate, probably distichous, glabrous, margins entire; venation brochidodromous. Inflorescences axillary or subterminal, racemose, 8- to 23-flowered, bracteate. Flowers hermaphroditic, transversely ellipsoid (in bud), dorsiventrally flattened, (4)5(6)-merous, epigynous, actinomorphic, pedicellate; calyx absent; petals (4)5(6), free, valvate; stamens (4)5(6), oppositipetalous (adnate near base of petal lobe and below the disk), introrse, dorsibasifixed; anthers tetrasporangiate (two pairs of divaricate locules), each loculus of a theca opening by a common, longitudinal slit; nectary disk suborbicular, fleshy, located between stamen and stigma; disk lobes (4)5(6), entire, rounded, each lobe alternating with a filament; gynoecium embedded in nectary disk tissue; ovary inferior, 1-locular; placental column straight; ovules 1 to 3, apical, pendulous; style very short cylindric or absent; stigma subsessile, with 3 to 5 lobes. Fruits drupaceous, single-seeded, broadly transversely ellipsoidal to subspheroidal, glabrous, smooth, with persistent floral parts at apex; exocarp fleshy, thin; pyrene (i.e., seed plus mesocarp) broadly transversely ellipsoidal to subspheroidal, with a thin, bony, smooth or finely striate mesocarp; endocarp papyraceous, very thin. Seeds broadly transversely ellipsoidal; endosperm copious.

Etymology. The generic name honors Robert Knud Friedrich Pilger (1876–1953). In the early 1900s, Pilger published treatments of Santalaceae for the floras of New Caledonia and New Guinea (Pilger, 1906, 1908, 1924). He later wrote the treatment for Santalaceae for Engler and Prantl's *Die Natürlichen Pflanzenfamilien* (Pilger, 1935). Compared with the earlier work on the family in that series (Hieronymus, 1889), Pilger's treatment expanded Santalaceae by including six genera (all placed in his tribe, Osyrideae), and his observations have greatly improved our understanding of the relationships between Santalaceae genera. Three genera have previously been named in Pilger's honor, *Pilgerodendron* Florin (Cupressaceae), *Pilgerochloa* Eig (Poaceae), and *Pilgeria* Schmidle (a cyanobacterium).

Pilgerina madagascariensis Z. S. Rogers, Nickrent & Malécot, sp. nov. TYPE: Madagascar. Toliara Prov.: Mandena Forestry Station, parcel M7, littoral forest on white sand, 24°56′10″S, 47°01′55″E, 10 m, 19 Jan. 2006 (fl.), *Z. Rogers, R. Ranaivojaona, F. Randriatafika, J. Rabenantoandro, C. Davidson & S. Christoph 890* (holotype, MO!; isotypes, B!, BM!, BR!, CAS!, G!, K!, L!, LE!, MA!, MO[2]!, NSW!, NY!, P!, PE!, PRE!, TAN!, US!, WAG!). Figure 5.

Shrubs or trees to 12 m tall; branches ± zig-zag, glabrous; young branches angular; mature branches terete; bark smooth, exfoliating in strips on older growth. Leaves 4–13.2 × 1.2–4 cm, length:width ratio 3–4.5:1, glabrous; leaf blades elliptic to lanceolate, rarely ovate, glabrous, chartaceous or less often chartaceous-coriaceous, apex acute or slightly acuminate, tip usually rounded, margin revolute, base long attenuate or cuneate-attenuate; midrib raised on both surfaces; venation slightly raised on both surfaces; secondary veins 6 to 10 pairs per side; fine venation irregularly reticulate, loosely arranged, often inconspicuous; petioles 3–7 mm, glabrous, often weakly caniculate adaxially, rounded abaxially, articulate at base. Inflorescences 1–2 cm, 8- to 23-flowered; inflorescence axes ca. 1.5 mm diam. at base, sparsely puberulent to nearly glabrous, trichomes ca. 0.1 mm, erect or subadpressed; bracts triangular-ovate, 0.3–0.6 × 0.4–0.5 mm, clasping each pedicel base, often caducous before anthesis, glabrous or only sparsely puberulent along margin adaxially, sparsely to moderately puberulent abaxially, trichomes generally less than 0.1 mm, erect or subadpressed, more concentrated along margin and near base. Flowers ca. 1 mm (excluding the pedicel), ca. 5 mm diam., transversely ellipsoid, dorsiventrally flattened, green; pedicels 3–4 mm, sparsely puberulent, trichomes ca. 0.1 mm, erect or less often subadpressed; calyx absent; petals triangular-ovate, 1.8–2.5 × 1.5–2 mm, spreading (becoming reflexed near apex), pubescent behind filaments adaxially, those trichomes 0.5–1 mm, matted and wavy (trichomes weakly attached to the abaxial surface of the filament near the anther connective), sparsely puberulent or glabrescent abaxially, those trichomes 0.05–0.1 mm, erect or less often subadpressed, apex adaxially puberulent, margin puberulent; filaments 0.5–0.7 × 0.3–0.35 mm (at base), glabrous; anthers ca. 0.5 × 0.6 mm, glabrous; nectary disk 2.5–3 mm diam., fleshy, glabrous, green, shiny exudate observed on dry material; disk lobe ca. 0.3 × 1 mm; style very short cylindric to absent, ca. 0.1 × 0.3–0.4 mm; stigma depressed in the middle. Fruits drupaceous, broadly transversely ellipsoidal to subspheroidal, 1.2–1.9 cm (excluding pedicel), 1.7–2.7 cm diam., green, glabrous, only 1 or 2 developing per inflorescence, base attenuate with pedicel; fruiting pedicels 1–10.5 mm, often elongating substantially, 1.5–2.5 mm diam., glabrous; exocarp fleshy, 0.5–2 mm thick; mesocarp bony, ca. 0.5 mm thick, smooth to finely striate; endocarp papyraceous, ca. 0.1 mm thick. Seeds broadly transversely ellipsoidal to subspheroidal, 1.2–1.4 cm, 1.4–1.7 cm diam.; embryo 7–8 mm, incurved.

Distribution and phenology. *Pilgerina madagascariensis* is a widespread but patchily distributed Malagasy endemic occurring between 0 and 1200 m elevation (Fig. 3). Populations are known from several small littoral forest fragments (Mandena, Sainte Luce) in the extreme southeast of the island on sand, in the drier forests (Ihosy, Isalo, Zombitse) of the west on sand and sandstone, in the humid forest of the central plateau (Andilamena, Lac Alaotra), and on the calcareous massif of Ankarana located in the north of the island. The species flowers from October through January and fruits from November through April.

Vernacular name. Sakaimboalavo (*J. Rabenantoandro et al. 315A*; *Z. Rogers et al. 890, 976*).

Conservation status. *Pilgerina madagascariensis* has been collected inside five formally protected areas (Ankarana, Isalo, Mandena, Sainte Luce, Zombitse). The species has an EOO of 230,000 km^2 and an AOO of 70,000 km^2 based on a 100-km^2 grid cell size. The species is assigned a preliminary IUCN conservation status of Least Concern (LC) (IUCN, 2001).

Paratypes. MADAGASCAR. **Antsiranana Prov.:** Ankarana, ouest (Nord), plateau de l'Ankarana, environs sud de Mahamasina (Antanatsimanaja), [12°54′S, 49°08′E], *Service Forestier (Capuron) 23390* (G, K, MO, P, TEF). **Fianarantsoa Prov.:** Isalo, plateaux et vallées de l'Isalo, à l'Ouest de Ranohira, [22°24′S, 45°17′E], *H. Humbert 28660* (P); Menarahaka, ouest (confins du centre), dans le

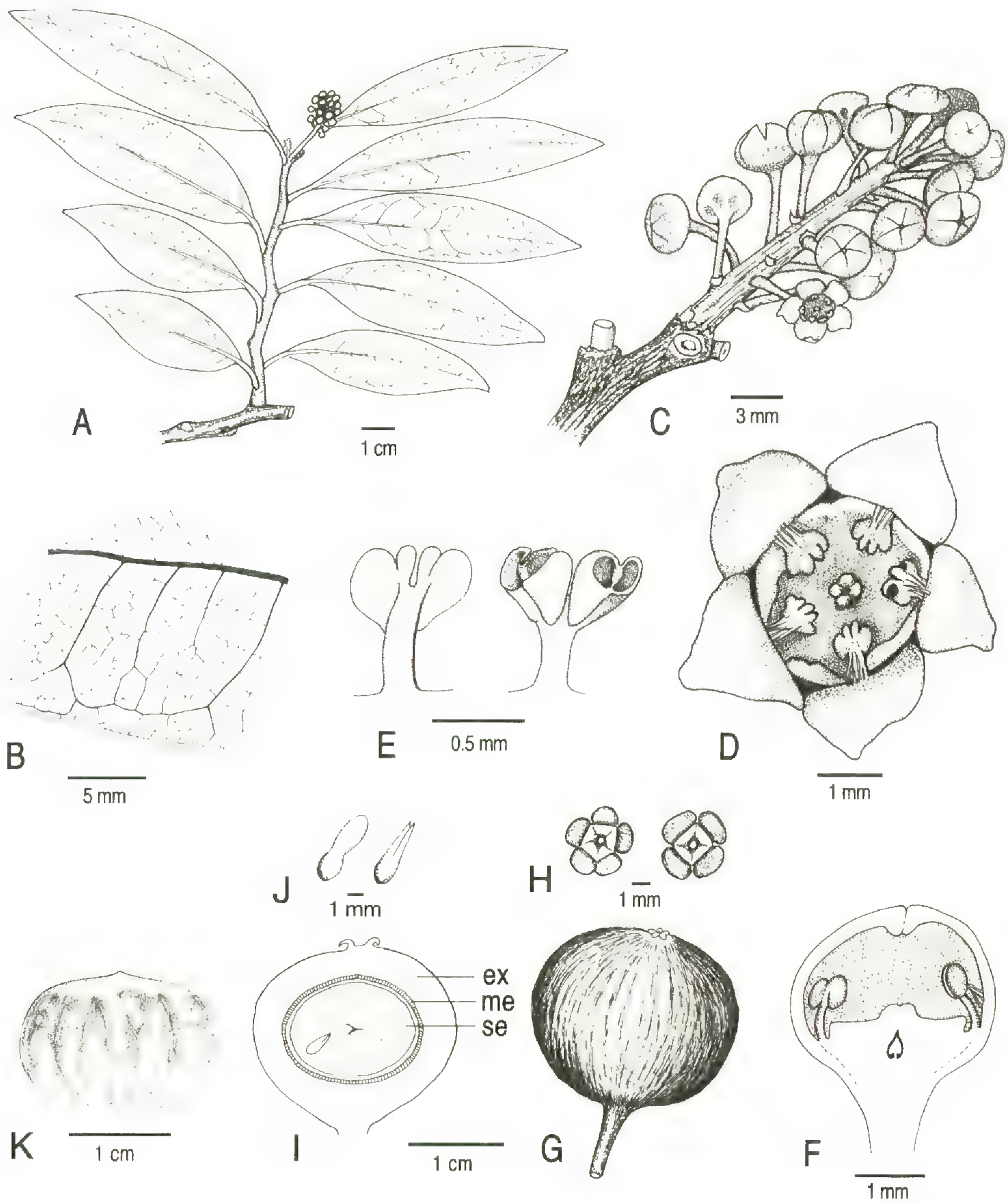

Figure 5. *Pilgerina madagascariensis*. —A. Flowering branch. —B. Cleared leaf showing venation pattern. —C. Racemose inflorescence. —D. Open flower, viewed from top. —E. Stamens from back (left) and front (right). —F. Bud in longitudinal section showing free-central pendulous placentation. —G. Fruit, lateral view. —H. Apex of fruit showing persistent perianth lobes, disk, and style in 5- (left) and 4-merous (right) flowers. —I. Longitudinal section of fruit; ex = exocarp, me = mesocarp, se = seed endosperm. —J. Detail of embryos, lateral and side views. —K. Surface view of pyrene (mesocarp plus enclosed seed). A–F drawn from *J. Rabenantoandro et al. 3154* (MO), G–K drawn from *H. Humbert 28660* (P).

bassin de la Menarahaka, près du carrefour des routes d'Ihosy à Ivohibe et Iakora, [22 32′S, 46 30′E], *Service Forestier (Capuron) 22622* (MO, P, TEF). **Toamasina Prov.:** Ambodisaina, forêt degradée, 17 17′25″S, 18 10′30″E, *S. Rakotonandrasana 627* (MO); Andilamena, Fivondronona Andilamena, Commune Andilamena, Fokotany Anony Ampamoha, forêt de Soavalivato route Vohitraivo-Andilamena, 17 12′12″S, 18 33′08″E, *R. Randrianaivo, A. Ratodimanana, T. Razafindrabeaza, H. Rajanatsoa, P. Rakotondramanza & O. Rabozanahary 825* (INH, K, MO, P, TAN); Lac Alaotra, Chutes du Maningory, rive gauche, [17 22′S, 48 18′E], *L. Homolle 531* (P). **Toliara Prov.:** Mandena, Fort Dauphin, rt. de Sainte Luce, forêt de Mandena, forêt sèche littorale, 24 57′S, 47 00′E, *L. Allorge & C. Bourgeois 850* (P), *C. Bourgeois 36* (MO), *N. Dumetz 1235* (MO, P, TAN); Mandena, Forêt marécageuse, M7 à Mandena, Ampasy, Taolagnaro, 21 56′08″S, 47 01′48″E, *J. Rabenantoandro, R. Rabevohitra, L. Randrihasipara & E. Ramisy 3151* (INH, MO[2], P, TAN, TEF); Sainte Luce, N of Fort-Dauphin, NW of Sainte Luce village, 24°45′12″S, 47°10′25″E, *Z. Rogers, R. Ranaivojaona, E. Ramisy, C. Davidson & S. Christoph 976* (B, BR, CAS, K, L, MO[2], NSW, P[2], PE, TAN[2], TEF, US, WAG), *G. McPherson, N. Dumetz & R. Rabevohitra 11227* (B, MO, P, TAN, TEF), *G. McPherson, N. Dumetz & R. Rabevohitra 14843* (MO); Sakaraha, 18 km E of Sakaraha on rd. to Ihosy, 22 52′S, 44 41′E, *J. Miller & R. Keating 4532* (MO, P, TAN); Zombitse, Forêt de Besaka-Zombitsy, 15–20 km E of Sakahara, W of Tulear, on Rte. #7, 22 46′S, 44°40′E, *T. Croat 30716* (P).

Discussion

Features of the inflorescence and fruit indicate that *Staufferia* and *Pilgerina* share an affinity with a group of eight small Santalaceae genera previously recognized by Stauffer (1957), namely *Acanthosyris* (five to seven species, Costa Rica to northern Argentina; Nee, 1996; Ulloa Ulloa & Jørgensen, 2002), *Cervantesia* (one to four species, Andean Colombia, Bolivia, Ecuador, and Peru; Pilger, 1935; Stauffer, 1961; Nee, 1996; Ulloa Ulloa & Jørgensen, 2002), *Jodina* (one species, Bolivia, Brazil, Uruguay, and Argentina; Stauffer, 1961; Nee, 1996), *Scleropyrum* (four to six species, roughly India and China to Malesia and New Guinea; Macklin & Parnel, 2002; Nianhe & Gilbert, 2003), *Pyrularia* (two species, eastern United States and approximately India to China; Leopold & Muller, 1983; Nianhe & Gilbert, 2003), and *Okoubaka* (one to two species, Ivory Coast, Ghana, and Democratic Republic of Congo; Léonard & Troupin, 1950; Villiers, 1973a, b). This grouping of genera, never classified at a subfamilial level within the Santalaceae, was previously considered by Pilger (1935) to be part of Osyrideae, which is a heterogeneous assemblage of 21 genera (Der & Nickrent, 2008). All of these genera share drupaceous (pseudodrupaceous) fruits with bony mesocarps (not endocarps as sometimes erroneously reported; see Bhatnagar & Sabharwal, 1969), and most possess fruits larger than other members of Santalaceae (greater than 3 cm diam.), although fruit size in *Staufferia* and *Pilgerina* is smaller (less than 2 cm long). The genera of our *Cervantesia* clade (Fig. 1) have hermaphroditic flowers with half-inferior ovaries, whereas four of the five genera of the *Pyrularia* clade (Fig. 1) are dioecious with inferior ovaries (the exception being *Pilgerina* with hermaphroditic flowers). Features that appear diagnostic for the *Pyrularia* clade (vs. *Cervantesia* clade) are the straight (vs. contorted) placental columns, reflexed (vs. straight) petals at anthesis, and dorsibasifixed (vs. dorsifixed) anthers.

Relatively few autapomorphic (i.e., diagnostic) character states exist for any one genus in the group, and variation between genera generally involves quantitative and continuous character states. A cladistic analysis of morphological characters, such as those selected characters shown in Table 3, recovers a tree (Nickrent, unpublished data) that shares few features of the molecular tree (Fig. 1), thus suggesting that many of the morphological characters are homoplastic. Despite this, genera in the *Pyrularia* and *Cervantesia* clades are defined by unique combinations of character states. For example, Stauffer (1957) listed a number of diagnostic morphological features that distinguish *Okoubaka* from *Scleropyrum*, particularly inflorescence structure and fruit organization, which can be extended to differentiate all members of the *Pyrularia* clade, including our two newly named genera (Table 3).

Staufferia differs most notably from *Okoubaka*, the most morphologically similar genus, by the smaller inflorescences (4 to 10 vs. 50 to 100 flowers); smaller (1.8–2 × [1.1–]1.4–1.6 cm vs. ca. 9 × 5 cm), obovoidal (vs. ellipsoidal) fruits; smaller (1.1–1.6 cm vs. > 3 cm diam.), obovoidal (vs. ellipsoidal) pyrenes (i.e., mesocarp plus the enclosed seed); smaller (ca. 1.5 mm vs. 7–8 mm diam.) persistent perianth; thinner (0.5–1 mm vs. 15–20 mm thick), 5-sulcate (vs. smooth) exocarp; and by the thinner (ca. 0.5 mm vs. 3–4 mm thick), smooth (vs. deeply striate or alveolate) mesocarp. *Pilgerina* is distinguished from *Scleropyrum* by the smaller inflorescences (8 to 23 vs. 60 to 100 flowers); pedicellate (vs. sessile) flowers; smaller (1.2–1.9 × 1.7–2.7 cm vs. ca. 3 × 2 cm), broadly transversely ellipsoidal to subspheroidal (vs. obovoidal to pyriform) fruits; broadly transversely ellipsoidal to subspheroidal (vs. subspheroidal to obovoidal) pyrenes; and by the thinner (ca. 0.5 mm vs. 1.5–3 mm thick), smooth or finely striate (vs. deeply striate or alveolate) mesocarp.

An argument could be made to lump instead of split with regard to placement of these two new taxa in distinct novel genera within the Santalaceae. Clearly both are related to the three genera of the *Pyrularia* clade, but given the topology of the phylogenetic tree (Fig. 1), retaining monophyly would require several unfavorable taxonomic transfers. If *Staufferia* were considered congeneric with *Scleropyrum*, the two species of *Okoubaka* would also have to be included, resulting in an extremely morphologically heterogeneous *Scleropyrum*. A more radical approach would be to lump *Pilgerina*, or even all five genera, into *Pyrularia* (the oldest name, 1803), but this would only exacerbate the problem of heterogeneity. As will be shown below, the genera of the *Pyrularia* clade are mutually as distinct, both by morphological and molecular characters, as other genera in Santalaceae, thus their classification at the generic rank is not incompatible with the existing classification.

When using molecular data to delimit genera within families, workers usually examine phylogenetic trees, identify monophyletic and diagnosable clades, and then name these clades (genera) with the goal of minimizing disruption of existing nomenclature. This approach was recently used to justify recircumscription of genera that display polyphyly and paraphyly (e.g., Kellermann et al., 2005; Pfeil & Crisp, 2005; Alejandro et al., 2005). In the latter study, the genus *Mussaenda* L. (Rubiaceae) and relatives in tribe Mussaendeae were recently reclassified based on

Table 3. Comparison of some selected morphological features among nine exemplar species of the *Pyrularia* and *Cervantesia* clades. Table based on a matrix of morphological characters (Nickrent, unpublished data).

Taxon	Floral bract	Pedicel	Anther attachment	Placental column	Fruit diam. (cm)	Exocarp surface	Fruit indument	Pyrene shape	Exocarp thickness (mm)	Mesocarp thickness (mm)	Mesocarp ornamentation
Acanthosyris asipapote	persistent	absent or subsessile	dorsifixed	twisted	> 3	smooth	glabrous	ellipsoidal	1	3–4	smooth
Cervantesia tomentosa	caducous	absent	dorsifixed	twisted	< 3	5-valved (basally and apically dehiscent from fruit)	glabrescent	ellipsoidal-spheroidal	0.3	0.2	smooth
Jodina rhombifolia	persistent	absent	dorsifixed	twisted	< 3	5-valved (basally and apically dehiscent from fruit)	pubescent	ellipsoidal-spheroidal	0.5–1	0.1–0.2	smooth
Okoubaka aubrevillei	persistent	present	dorsibasifixed	straight	> 3	smooth	glabrous	ellipsoidal	15–20	3–4	deeply striate or alveolate
Pilgerina madagascariensis	persistent	present	dorsibasifixed	straight	< 3	smooth	glabrous	broad transversally ellipsoidal to subspheroidal	0.5–2	0.5	smooth or finely striate
Pyrularia pubera	caducous	present	dorsibasifixed	straight	< 3	smooth	glabrous or sparsely pubescent at least near apex	obovoidal	0.5–1	0.5–1	smooth or verrucose
Scleropyrum pentandrum	persistent	absent	dorsibasifixed	straight	< 3	smooth	glabrous	subspheroidal to obovoidal	1–3	1.5–3	deeply striate or alveolate
Staufferia capuronii	caducous	present or absent	dorsibasifixed	straight	< 3	5-fused segments (not dehiscent)	pubescent or glabrescent	obovoidal	0.5–1	0.5	smooth

molecular data. Combined *trnT-F* and ITS data were used to justify lumping *Aphaenandra* Miq. into *Mussaenda*, maintaining *Pseudomussaenda* Wernham as a distinct genus and renaming a clade (composed of both *Landiopsis* Capuron ex Bosser and *Mussaenda* p.p.) as a new genus *Bremeria* Razafim. & Alejandro. Unlike these analyses, however, genera of the *Pyrularia* and *Cervantesia* clades are monotypic or small, with each member recognized by distinct diagnostic inflorescence and fruit features (Table 3; see also Stauffer, 1957).

Molecular data have also been used to show that the taxon "genus" is not equivalent within vertebrates (Johns & Avise, 1998) and euascomycetes (Lumbsch, 2002), and it is reasonable to extrapolate this finding to other groups, including angiosperms. The reasons for inequivalency were summarized by Lumbsch (2002), as were the effects this has on nomenclature, specifically the ongoing debate between those advocating the use of Linnaean ranks versus rankless systems that utilize phylogenetic principles. Our approach in this paper is essentially a combination of these two philosophies. By naming two new monospecific genera, we demonstrate our recognition of this rank, while at the same time we adhere to the concept of monophyly. Moreover, because molecular data are available for these taxa, we are compelled to utilize intergeneric genetic distances to maintain internal consistency in our classification.

Using our four-gene matrix, uncorrected "p" distances were calculated using PAUP* (Swofford, 2002) for all pairs of Santalaceae taxa (other models of molecular evolution, including the general time reversible, gave comparable results). The average *P* value between the *Pyrularia* and *Cervantesia* clades was 0.039. Within the *Pyrularia* clade, intergeneric distances averaged 0.0165, whereas within the *Cervantesia* clade the average was 0.0125. The latter changed little whether one or both species of *Acanthosyris* were included. Thus, it appears that there is greater differentiation between Old World genera than between New World genera, at least for the genes used in this study.

As a reflection of the lower level of generic distinctiveness of *Cervantesia* and *Acanthosyris*, it can be pointed out that several species have been transferred from the former to the latter genus: *C. colombiana* A. C. Sm. (Smith, 1937) to *A. colombiana* (A. C. Sm.) Cuatrec. by Cuatrecasas (1950) and *C. glabrata* Stapf (Stapf, 1906) to *A. glabrata* (Stapf) Stauffer by Stauffer (1961). The average intergeneric distances within the *Pyrularia* clade ranged from 0.0136 (*Staufferia*) to 0.0188 (*Okoubaka*) with a mean across all genera of 0.0165. The distance for *Pilgerina* (0.0176) was greater than the mean distance values for both the *Pyrularia* clade and the *Cervantesia* clade (0.0125). This approach provides additional, internally consistent genetic data that justify recognizing these taxa as new monospecific genera, a result that is not surprising given the level of endemism of angiosperm taxa on Madagascar.

Our phylogeny suggests that in the *Pyrularia* clade, the North American taxa are sister to an Afro-Asiatic clade, whereas the Malagasy taxa form a paraphyletic group. Using the age estimation of Malécot (2002), the divergence between *Pyrularia* and other taxa occurred during Middle Eocene, while the split of *Okoubaka* and *Scleropyrum* took place sometime in the Late Eocene. Given this, the speciation of the two Malagasy genera may have occurred between the Middle and Late Eocene, an age consistent with divergence dates derived in other groups (e.g., cichlid fish [Yoder et al., 1996], lemurs [Vences et al., 2001], chameleons [Raxworthy et al., 2002], and *Acridocarpus* Guill. & Perr. in the Malpighiaceae [Davis et al., 2002]).

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A REVISION OF THE *SOLANUM HAVANENSE* SPECIES GROUP AND NEW TAXONOMIC ADDITIONS TO THE GEMINATA CLADE (*SOLANUM*, SOLANACEAE)[1]

Sandra Knapp[2]

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Abstract

Recent studies of *Solanum* L. (Solanaceae) phylogeny using both plastid and nuclear DNA sequences have shown that a distinct clade comprising the members of *Solanum* sect. *Geminata* (G. Don) Walp. (woody, non-spiny solanums with simple or branched trichomes and usually leaf-opposed inflorescences) also includes a small Caribbean group of species known as section *Diamonon* (Raf.) A. Child, whose relationships have previously been obscure: *S. acropterum* Griseb., *S. conocarpum* Dunal, *S. havanense* Jacq., and *S. troyanum* Urb. Work on a monograph of section *Holophylla* (G. Don) Walp. (s.l.) has also revealed that several species previously excluded from section *Geminata* on morphological grounds belong to the more broadly circumscribed group recognized as the Geminata clade. In addition, since the publication of the monograph of *Solanum* sect. *Geminata*, seven new species of these rare, forest understory shrubs have been described (*S. chalmersii* S. Knapp, *S. humboldtianum* Granados-Tochoy & S. Knapp, *S. monanthemon* S. Knapp, *S. naucinum* S. Knapp, *S. pseudodaphnopsis* L. A. Mentz & Stehmann, *S. sagittantherum* Granados-Tochoy & C. I. Orozco, and *S. sumacaspi* S. Knapp). An additional new species from Ecuador, *S. elvasioides* S. Knapp, is described here. I review the status and circumscription of section *Geminata* in the New World and its relation to the Geminata clade, and provide descriptions for all the species of the Caribbean *S. havanense* species group and all species previously excluded from section *Geminata*. Lectotypes are selected for *S. acropterum*, *S. hookerianum* A. Spreng., *S. myrtifolium* Lodd., *S. havanense* Vell., *S. troyanum*, *S. argentinum* Bitter & Lillo, and *S. evonymoides* Sendtn., and a neotype is selected for *S. havanense*.

Key words: Caribbean, IUCN Red List, Neotropics, section *Diamonon*, section *Geminata*, Solanaceae, *Solanum*.

The Solanaceae is an economically important, cosmopolitan family with approximately 3000 species in some 90 genera. The family has members occurring in all habitats, from the driest deserts on the western coasts of South America to the dense, wet, tropical rainforest of the Amazon and Southeast Asia. Life

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forms in the family range from canopy trees to minute ephemeral herbs, with enormous variation in between. Members of the family include globally important food crops such as potatoes and tomatoes (*Solanum tuberosum* L. and *S. lycopersicum* L., respectively) and a number of widely used drug plants such as *Nicotiana* L., the source of tobacco, and *Atropa* L., the source of atropine. Approximately half of the species in the family are contained in five genera, the largest and most diverse of which is *Solanum* L. (Knapp et al., 2004).

Solanum is one of the 10 most species-rich genera of flowering plants (Frodin, 2004). With approximately 1500 species (J. Bennett & S. Knapp, unpubl.) occurring on all temperate and tropical continents, the genus occupies an incredibly wide range of habitats and habits, paralleling that of the family. The history of *Solanum* classification has been reviewed in Knapp (2002a), but the last time the genus was monographed in its entirety was in de Candolle's *Prodromus* (Dunal, 1852). Current work by participants of the "PBI *Solanum*" project (see <http://www.nhm.ac.uk/solanaceaesource>) will result in a modern monographic treatment of the entire genus available online. *Solanum* can be divided into 13 well-supported monophyletic clades (Bohs, 2005; Weese & Bohs, 2007).

Solanum sect. *Geminata* (G. Don) Walp. is one of the largest sections of the extremely large plant genus *Solanum*. In Dunal's (1852) treatment of the group, he included 37 species (the history of classification and species description in section *Geminata* is reviewed in Knapp, 2002a). Knapp (2002a) included 126 species in section *Geminata*, defined as woody plants with leaf-opposed inflorescences, white flowers, and green fruits at maturity. These species have been considered difficult and are very similar morphologically; they have been described as "remarkably uniform on the herbarium sheet" (D'Arcy, 1973: 740), and Morton (1944: 54) described a new taxon (*S. orygale* C. V. Morton, a synonym of *S. arboreum* Dunal) as "having no outstanding features, but does not agree with any other species." Phylogenetic studies of *Solanum* using chloroplast and nuclear DNA sequences (Bohs, 2005; Weese & Bohs, 2007) have shown that the members of section *Geminata* (sensu Knapp, 2002a) form one of the 13 major clades in the genus, if a small group of endemic Caribbean species (see below) and several Neotropical taxa previously excluded from the section are included. Bohs (2005) called this larger, more inclusive monophyletic group the Geminata clade. This clade is well supported (100% bootstrap support in a three-gene analysis, Weese & Bohs, 2007), and, with 144 species currently known (see Appendix 1), it is the largest major clade of non-spiny solanums.

Solanum havanense Jacq. and *S. conocarpum* Dunal, two Caribbean endemic species previously recognized as the monospecific *Solanum* sect. *Diamonon* (Raf.) A. Child or of unknown relationships, respectively, have been included in the most recent molecular analyses (Weese & Bohs, 2007; J. C. Granados-Tochoy & L. Bohs, pers. comm.). These species are sister taxa at the base of the Geminata clade and are, in turn, apparently sister to *Solanum* sect. *Geminata* as recognized by Knapp (2002a).

Solanum delitescens C. V. Morton, recognized as a member of *Solanum* sect. *Geminata*, but of uncertain relationships by Knapp (2002a), has also been shown to be firmly nested within the Geminata clade in molecular analyses (Weese & Bohs, 2007), confirming its relationships as suggested in the earlier monograph. I excluded a number of species from the original treatment of *Solanum* sect. *Geminata* (Knapp, 2002a) based on inflorescence morphology, which I considered to be similar to that found in species of *Solanum* sect. *Holophylla* (G. Don) Walp. (s. str., see Knapp, 1989). Phylogenetic analyses using DNA sequences (Bohs, 2005; Weese & Bohs, 2007) have also shown one of these species (*S. argentinum* Bitter & Lillo) to be nested well within the Geminata clade. Subsequent study of the rest of these taxa as part of the preparation of a monograph of *Solanum* sect. *Holophylla* has shown that based on morphology, these previously excluded species are members of an enlarged and redefined Geminata clade. In addition, seven new species of the Geminata clade have been described since the publication of the monograph of *Solanum* sect. *Geminata* (Knapp, 2002a). Of the additional species included in the more broadly defined Geminata clade, only *S. argentinum*, *S. havanense*, and *S. conocarpum* have been used in molecular phylogenetic analyses (Weese & Bohs, 2007; L. Bohs, pers. comm.), but based on overall morphology, all of the other species included here belong in the larger, monophyletic Geminata clade.

For this treatment, I am defining the Geminata clade as the larger group containing all the species treated in Knapp (2002a), the set of species related to *Solanum havanense*, those taxa excluded from *Solanum* sect. *Geminata* previously, and all the species described as members of the section since Knapp (2002a). The clade has few unambiguous morphological synapomorphies (Bohs, 2005), but its members are generally woody plants with simple leaves that are glabrous or have simple or branched (never stellate) trichomes, terminal or leaf-opposed inflorescences, and white or purple flowers. In my *Flora Neotropica* monograph (Knapp, 2002a), I divided the members of section *Geminata* treated into a set of putatively monophyletic species groups. Recent molecular work has shown that these groups are, for the most part, not monophyletic (J. C. Granados-Tochoy, pers. comm.),

but sampling for molecular analyses has been relatively sparse within the group, and groupings obtained are very poorly supported. Although these species groups may not be monophyletic, they serve as initial, pragmatic groupings to assess relationships and aid in identification. To that end, I place each of the species described here in one of the species groups from Knapp (2002a) in which it would belong (Table 1), but in the descriptions I also elaborate on other similar or sympatric species from the entire clade. If these species groups, or the monophyletic groupings recovered from further analysis, were to be given sectional status (as has been done for the tomatoes and their relatives; see Peralta et al., 2008), the name *Solanum* sect. *Geminata* would apply to the grouping containing *S. nudum* Dunal, the type species of the section. In light of the only preliminary phylogenetic data available for the group, I am not formally recognizing any of these groups at sectional level, but continue to use a pragmatic set of species groups (sensu Whalen, 1989) in order to aid communication and further work with this, the largest of the clades of non-spiny solanums.

Within the species of the Geminata clade as treated here, trichome morphology and inflorescence structure are both extremely variable attributes. Variation occurs within species, populations, and even individuals in the degree of trichome branching, which traditionally has been one of the most important characters used in *Solanum* taxonomy (see Knapp, 2002a). Section *Geminata*, as previously treated (Knapp, 2002a), includes species with simple trichomes and species with branched trichomes, a conclusion strongly supported by recent molecular analyses (Bohs, 2005) for the Geminata clade as a whole. As I have studied the members of the Geminata clade in its broadest sense, it has become clear that, although useful for identification purposes, trichome morphology is not likely to be a particularly reliable synapomorphy for monophyletic groups.

Although species of the Geminata clade are distributed primarily in the New World tropics, a single species (*Solanum spirale* Roxb.) is found in forest habitats in the Old World, from southern China to Australia. As for *Solanum* as a whole (see Knapp, 2002b), the species richness of the Geminata clade is circum-Amazonian, although some species are found in the Amazon Basin. Highest numbers of species are found in Colombia and Peru (with 41 species each), but the highest endemism is in Brazil, where 17 of the 34 species occurring there are endemic, most of these to southeastern Brazil (see Table 2).

In this paper, I provide descriptions for the additional species shown by molecular analyses to be members of the Geminata clade (see above), species previously excluded from the group by Knapp (2002a), and taxa in the group described since Knapp (2002a) (see Appendix 2). I also provide preliminary conservation assessments for each of the species treated here, in order to contribute to Target 2 (a preliminary conservation assessment of all known plant species) of the *Global Strategy for Plant Conservation* (Secretariat of the Convention on Biological Diversity, 2002).

The *Solanum havanense* Species Group

Shrubs or trees; stems glabrous or minutely pubescent with uniseriate trichomes, these often white; new growth glabrous or minutely pubescent; sympodial units plurifoliate. Leaves simple, elliptic to narrowly elliptic, glabrous. Inflorescences terminal or internodal, often borne on short shoots, simple or branched, usually glabrous or minutely pubescent with simple trichomes. Flowers white or purple, relatively large, to 6 cm diameter, expanding during anthesis, the corolla lobes planar at anthesis. Fruit ellipsoid, dark green or blue-green to purple; fruiting pedicels erect or deflexed; seeds rounded or ovoid, dark brown.

Distribution. All species are endemic to islands in the Caribbean, from coastal and montane forests.

Members of what I am calling here the *Solanum havanense* species group (also see Knapp, 2002a) have long been of uncertain affinity within *Solanum*. Rafinesque (1836: 76) based his genus *Diamonon*, with the single species *D. coriaceum* (Hook.) Raf., on the possession of a "constantly and unequally four-partite" (zygomorphic) calyx shown in the plate of *S. coriaceum* Hook. (Hooker, 1827). Although Hooker (1827: text opposite plate 2708) stated that flowers were "often with an irregularity" in the corolla lobes, this is not apparent from the plate in his publication. The calyx structure as depicted in Hooker's plate (see Fig. 4) shows a five-parted calyx with two of the lobes fused at the margin, a condition common in *Solanum*, where the calyx lobes do not split regularly at corolla egress (see description of *S. havanense* below). Subsequent authors (Child, 1998), not recognizing the relationship of *S. havanense* to the other species included here, considered section *Diamonon* as a distinct, monospecific group at the sectional level (as *Solanum* sect. *Diamonon*) within *Solanum*. The inflorescences borne on short shoots and large purple flowers led Nee (in litt., October 1989) to consider *S. havanense* a species of *Lycianthes* (Dunal) Bitter; closer examination of morphology coupled with molecular data (Bohs, 2005) clearly place it in the Geminata clade of *Solanum*. Two species of the group (*S. havanense* and *S. conocarpum*) have been included in the most recent molecular analysis (J. C. Granados-Tochoy & L. Bohs,

Table 1. Species groups in the Geminata clade (Knapp [2002a] and species treated here) using the species group names established by Knapp (2002a); several taxa are treated here as incertae sedis. They are clearly members of the Geminata clade but with no clear affinities to any of the pragmatic species groups listed here. Species treated in this paper are in boldface.

***Solanum havanense* species group**
***Solanum acropterum* Griseb.**
***Solanum conocarpum* Dunal**
***Solanum havanense* Jacq.**
***Solanum troyanum* Urb.**
Solanum oblongifolium species group
Solanum clivorum S. Knapp
Solanum hypaleurotrichum Bitter
Solanum oblongifolium Dunal
Solanum venosum Dunal
***Solanum pseudocapsicum* species group**
***Solanum argentinum* Bitter & Lillo**
***Solanum delicatulum* L. B. Sm. & Downs**
Solanum diphyllum L.
Solanum kleinii L. B. Sm. & Downs
Solanum malacothrix S. Knapp
Solanum pseudocapsicum L.
Solanum spissifolium Sendtn.
***Solanum nudum* species group**
Solanum acuminatum Ruiz & Pav.
Solanum aphyodendron S. Knapp
Solanum caavurana Vell.
Solanum campaniforme Roem. & Schult.
Solanum cassioides L. B. Sm. & Downs
***Solanum chalmersii* S. Knapp**
Solanum cordioides S. Knapp
Solanum daphnophyllum Bitter
Solanum gertii S. Knapp
Solanum intermedium Sendtn.
Solanum lasiopodium Dunal
***Solanum monanthemon* S. Knapp**
Solanum nudum Dunal
***Solanum pabstii* L. B. Sm. & Downs**
Solanum pseudoquina A. St.-Hil.
Solanum reitzii L. B. Sm. & Downs
Solanum restingae S. Knapp
Solanum santosii S. Knapp
Solanum spirale Roxb.
Solanum symmetricum Rusby
Solanum tepuiense S. Knapp
Solanum trachytrichium Bitter
Solanum trichoneuron Lillo
Solanum warmingii Hieron.
***Solanum leucocarpon* species group**
Solanum alatirameum Bitter
Solanum corumbense S. Moore
***Solanum evonymoides* Sendtn.**
Solanum leucocarpon Dunal
Solanum lindenii Rusby
Solanum oblongum Ruiz & Pav.
Solanum nutans species group
Solanum malletii S. Knapp

Table 1. Continued.

Solanum microleprodes Bitter
Solanum nutans Ruiz & Pav.
Solanum xanthophaeum Bitter
Solanum youngii S. Knapp
***Solanum amblophyllum* species group**
Solanum amblophyllum Hook.
Solanum barbulatum Zahlbr.
***Solanum elvasioides* S. Knapp**
Solanum laurifrons Bitter
Solanum psychotrioides Dunal
Solanum tovarii S. Knapp
Solanum vacciniiflorum Standl. & L. O. Williams
Solanum validinervium Benítez & S. Knapp
Solanum deflexiflorum species group
Solanum deflexiflorum Bitter
Solanum imberbe Bitter
Solanum incomptum Bitter
Solanum irregulare C. V. Morton
Solanum sieberi Van Heurck & Müll. Arg.
***Solanum arboreum* species group**
Solanum amnicola S. Knapp
Solanum anisophyllum Van Heurck & Müll. Arg.
Solanum arboreum Dunal
Solanum falconense S. Knapp
Solanum goniocaulon S. Knapp
Solanum gratum Bitter
***Solanum humboldtianum* Granados-Tochoy & S. Knapp**
Solanum laevigatum Dunal
Solanum lucens S. Knapp
Solanum ploumanii S. Knapp
Solanum ramonense C. V. Morton & Standl.
Solanum ripense Dunal
Solanum roblense Bitter
***Solanum sagittantherum* Granados-Tochoy & C. I. Orozco**
Solanum tanysepalum S. Knapp
Solanum unifoliatum species group
Solanum bahianum S. Knapp
Solanum bellum S. Knapp
Solanum cyclophyllum S. Knapp
Solanum longevirgatum Bitter
Solanum marantifolium Bitter
Solanum triplinervium C. V. Morton
Solanum unifoliatum S. Knapp
Solanum robustifrons species group
Solanum abitaguense S. Knapp
Solanum cucullatum S. Knapp
Solanum heleonastes S. Knapp
Solanum robustifrons Bitter
Solanum superbum S. Knapp
Solanum narcoticosmum species group
Solanum dasyneuron S. Knapp
Solanum foetens S. Knapp
Solanum narcoticosmum Bitter
***Solanum nigricans* species group**
Solanum callianthum C. V. Morton
***Solanum canoasense* L. B. Sm. & Downs**

Table 1. Continued.

Solanum cornifolium Dunal
Solanum maturecalvans Bitter
Solanum nigricans M. Martens & Galeotti
Solanum ochrophyllum Van Heurck & Müll. Arg.
Solanum platycypellon S. Knapp
Solanum quebradense S. Knapp

***Solanum arenarium* species group**
Solanum arenarium Sendtn.
***Solanum compressum* L. B. Sm. & Downs**
Solanum gnaphalocarpon Vell.
***Solanum pseudodaphnopsis* L. A. Mentz & Stehmann**
Solanum smithii S. Knapp
Solanum tunariense Kuntze

Solanum sessile species group
Solanum chlamydogynum Bitter
Solanum confertiseriatum Bitter
Solanum monadelphum Van Heurck & Müll. Arg.
Solanum obovalifolium Pittier ex Benítez
Solanum oppositifolium Ruiz & Pav.
Solanum palmillae Standl.
Solanum rovirosanum Donn. Sm.
Solanum sessile Ruiz & Pav.
Solanum triste Jacq.
Solanum turgidum S. Knapp

Solanum confine species group
Solanum capillipes Britton
Solanum confine Dunal
Solanum cruciferum Bitter
Solanum darienense S. Knapp
Solanum dissimile C. V. Morton
Solanum erosomarginatum S. Knapp
Solanum hypocalycosarcum Bitter
Solanum leptopodum Van Heurck & Müll. Arg.
Solanum leptorhachis Bitter
Solanum morii S. Knapp
Solanum nematorhachis S. Knapp
Solanum ombrophilum S. Knapp
Solanum pastillum S. Knapp
Solanum pertenue Standl. & C. V. Morton
Solanum stipulatum Vell.
Solanum tenuiflagellatum S. Knapp
Solanum tuerckheimii Greenm.
Solanum valerianum C. V. Morton & Standl.
Solanum yanamonense S. Knapp

***Solanum dolosum* species group**
Solanum dolosum C. V. Morton ex S. Knapp
Solanum gonyrhachis S. Knapp
Solanum habrocaulon S. Knapp
***Solanum naucinum* S. Knapp**

Taxa incertae sedis
Solanum delitescens C. V. Morton
Solanum lasiocladum S. Knapp
Solanum mapiricum S. Knapp
***Solanum sumacaspi* S. Knapp**

pers. comm.), and they are resolved as sister taxa at the base of the Geminata clade and are, in turn, apparently sister to *Solanum* sect. *Geminata* as recognized by Knapp (2002a).

Preliminary conservation assessments are given here for the members of the *Solanum havanense* group in advance of a paper outlining the specific criteria for formal IUCN listing for all endemic Caribbean species of *Solanum* (Yoder et al., in prep.).

Key to the Species of the *Solanum havanense* Species Group

1a. Flowers white; pedicels strongly winged . . . . . *S. acropterum*
1b. Flowers purple; pedicels not winged.
  2a. New growth completely glabrous; leaves drying dark; flowers 3.5–6 cm diam. . . . . *S. troyanum*
  2b. New growth pubescent with white trichomes, these usually simple but sometimes dendritic.
    3a. Berry erect, dark purplish black, fleshy; anther pores minute, never splitting to slits . . . . . *S. havanense*
    3b. Berry pendent, green, hard and woody; anther pores lengthening to slits with age . . . . . *S. conocarpum*

1. Solanum acropterum Griseb., Fl. Brit. W.I. [Grisebach] 437. 1862. TYPE: Jamaica. s. loc., s.d., *J. Waters s.n.* (lectotype, designated here, K 000005250!). Figure 1.

Small trees to 10 m; stems erect, completely glabrous; bark pale gray-brown when young, darker red on older stems; new growth glabrous; sympodial units plurifoliate, appearing difoliate, but the inflorescence one per stem on short shoots. Leaves (2)5.5–14 × (1)3–5.5 cm, elliptic, glabrous on both surfaces, when dry densely white-spotted with crystal sand (oxalate inclusions), darker green adaxially (fide *Stearn 499*), the base acute to somewhat attenuate, the margins entire, the apex acute, rounded at the very tip; primary veins 5 to 8 pairs, drying yellow on older leaves, reddish orange on young leaves; petiole 1–2.5 cm, glabrous, markedly corrugated. Inflorescences leaf-opposed and on short leafy shoots, 2–6 cm, branching 1 to 3 times, with 3 to 15 flowers, completely glabrous, the peduncle almost absent to 2 cm; pedicels 15–20 mm, strongly and conspicuously 5-winged from the midrib of the calyx lobes, glabrous, nodding at anthesis, ca. 5 mm diam. at the apex, ca. 1 mm diam. at the base, the wings to 1.5 mm wide, articulated at the base; pedicel scars irregularly spaced 1–2 mm apart, the pedicels articulating slightly above the base leaving a peg 1–3 mm on the inflorescence; buds ellipsoid, the corolla ca. 1/2 of the way exserted from the calyx tube, included in the lobes. Flowers apparently all perfect; calyx tube 3–

Table 2. Geographical distribution of the Neotropical members of the Geminata clade (Knapp, 2002 and the species treated here) by country. Countries are listed in alphabetical order, followed by the number of endemics/total number of species. Country-endemic species are in boldface. *Solanum spirale* is the only exclusively Old World member of the group; its distribution is given in Knapp (2002a).

Country (endemics/total)	Species of the Geminata clade
Argentina (1/14)	argentinum, caavurana, compressum, corumbense, delicatulum, **delitescens**, heleonastes, maturecalvans, platycypellon, pseudocapsicum, pseudoquina, symmetricum, trachytrichium, trichoneuron
Belize (0/4)	diphyllum, nudum, rovirosanum, tuerckheimii
Bolivia (6/26)	acuminatum, aphyodendron, argentinum, **chalmersii**, confine, corumbense, daphnophyllum, **gonyrhachis**, heleonastes, leucocarpon, lindenii, **mapiricum**, maturecalvans, **monanthemon**, nudum, nutans, ochrophyllum, oppositifolium, platycypellon, pseudocapsicum, robustifrons, sessile, **superbum**, symmetricum, trichoneuron, **tunariense**
Brazil (17/34)	**alatirameum**, anisophyllum, **arenarium**, **bahianum**, caavurana, campaniforme, **canoasense**, cassioides, compressum, **cordioides**, corumbense, delicatulum, **evonymoides**, **gertii**, **intermedium**, **kleinii**, leptopodum, leucocarpon, nudum, oppositifolium, **pabstii**, pseudocapsicum, **pseudodaphnopsis**, pseudoquina, **reitzii**, **restingae**, robustifrons, **santosii**, sessile, **spissifolium**, **stipulatum**, symmetricum, trachytrichium, **warmingii**
Chile (0/1)	pseudocapsicum
Colombia (9/41)	aphyodendron, arboreum, barbulatum, callianthum, cornifolium, cyclophyllum, darienense, deflexiflorum, dissimile, dolosum, **humboldtianum**, imberbe, **irregulare**, laevigatum, lasiopodium, **laurifrons**, leptopodum, leptorhachis, leucocarpon, **longevirgatum**, lucens, malletii, marantifolium, microleprodes, **nematorhachis**, nudum, nutans, oblongifolium, oppositifolium, pseudocapsicum, **psychotrioides**, ripense, rovirosanum, **sagittantherum**, sessile, sieberi, **triplinervium**, **unifoliatum**, vacciniiflorum, validinervium, venosum
Costa Rica (0/15)	aphyodendron, arboreum, diphyllum, imberbe, incomptum, narcoticosmum, nudum, pastillum, pertenue, ramonense, roblense, rovirosanum, tuerckheimii, vacciniiflorum, valerianum
Cuba (0/2)	havanense, nudum
Dominica (0/2)	nudum, triste
Dominican Republic (0/1)	nudum
Ecuador (3/35)	abitaguense, anisophyllum, barbulatum, bellum, confertiseriatum, confine, cornifolium, corumbense, cruciferum, cucullatum, cyclophyllum, deflexiflorum, dolosum, goniocaulon, **elvasioides**, **hypaleurotrichum**, **hypocalycosarcum**, leptopodum, leptorhachis, leucocarpon, malletii, marantifolium, maturecalvans, nudum, nutans, oblongifolium, oppositifolium, plowmanii, pseudocapsicum, robustifrons, sessile, smithii, triplinervium, venosum, youngii
El Salvador (0/3)	aphyodendron, diphyllum, nudum
French Guiana (0/4)	campaniforme, leucocarpon, morii, oppositifolium
Grenada (0/1)	nudum
Guatemala (0/9)	aphyodendron, dasyneuron, diphyllum, narcoticosmum, nigricans, nudum, pseudocapsicum, rovirosanum, tuerckheimii
Guyana (0/3)	campaniforme, leucocarpon, oppositifolium
Haiti (0/1)	nudum
Honduras (0/4)	aphyodendron, diphyllum, nigricans, nudum
Jamaica (2/4)	**acropterum**, havanense, nudum, **troyanum**
Martinique (0/2)	sieberi, triste
Mexico (2/11)	aphyodendron, dasyneuron, diphyllum, **malacothrix**, narcoticosmum, nigricans, nudum, **palmillae**, pseudocapsicum, rovirosanum, tuerckheimii
Nicaragua (0/8)	aphyodendron, diphyllum, nudum, pastillum, pertenue, rovirosanum, tuerckheimii, valerianum
Panama (0/15)	aphyodendron, arboreum, darienense, imberbe, incomptum, leucocarpon, microleprodes, narcoticosmum, nudum, pertenue, ramonense, rovirosanum, tuerckheimii, vacciniiflorum, valerianum
Paraguay (0/10)	argentinum, caavurana, compressum, corumbense, delicatulum, heleonastes, pseudocapsicum, pseudoquina, symmetricum, trachytrichium

Table 2. Continued.

Country (endemics/total)	Species of the Geminata clade
Peru (11/41)	abitaguense, acuminatum, **amblophyllum**, **amnicola**, anisophyllum, aphyodendron, barbulatum, bellum, **clivorum**, confertiseriatum, confine, corumbense, cruciferum, cucullatum, daphnophyllum, goniocaulon, **habrocaulon**, laevigatum, leptopodum, leptorhachis, leucocarpon, lindenii, malletii, maturecalvans, **monadelphum**, **naucinum**, nudum, oblongifolium, **oblongum**, ochrophyllum, oppositifolium, plowmanii, pseudocapsicum, robustifrons, sessile, smithii, **sumacaspi**, **tovarii**, **xanthophaeum**, **yanamonense**, youngii
Puerto Rico (0/1)	nudum
Suriname (0/2)	leucocarpon, morii
Trinidad and Tobago (0/6)	arboreum, capillipes, nudum, pseudocapsicum, sieberi, triste
Uruguay (0/1)	pseudocapsicum
Venezuela (12/32)	aphyodendron, arboreum, campaniforme, capillipes, **chlamydogynum**, cornifolium, dissimile, **erosomarginatum**, **falconense**, **foetens**, **gratum**, imberbe, lasiocladum, leucocarpon, **lucens**, morii, nudum, oblongifolium, **obovalifolium**, ombrophilum, oppositifolium, pseudocapsicum, **quebradense**, ripense, sessile, sieberi, **tanysepalum**, **tenuiflagellatum**, **tepuiense**, triste, **turgidum**, validinervium
Virgin Islands (1/2)	**conocarpum**, nudum

3.5 mm, cup-shaped, glabrous, the lobes 3–8 mm, narrowly triangular to lanceolate, winged abaxially, the wing continuing onto the pedicel, glabrous, the apex rounded; corolla 2–2.5 cm diam., white, lobed 1/3 to 1/2 of the way to the base, the lobes ca. 1 cm, deltate to narrowly deltate, campanulate at anthesis, minutely papillose with tangled white trichomes at the tips; filaments with the free portion 1.5–2 mm, the filament tube less than 0.5 mm, glabrous; anthers 4–5 × 1–1.5 mm, the dorsal face thickened and darker when dry, slightly rounded-sagittate at the base, poricidal at the tips, the pores lengthening to slits with age; ovary conical, glabrous, the style 8–9 mm, straight, glabrous, the stigma minutely capitate, the surface papillate. Fruit a globose berry, becoming ellipsoid, ca. 1.2 × 1 cm (immature), green; fruiting pedicels ca. 2 cm, ca. 1.5 mm diam. at the base, woody, pendent; seeds ca. 3 × 2 mm, rounded reniform (immature), dark brown, the surfaces minutely reticulate-pitted. Chromosome number not known.

Distribution. Endemic to Jamaica, where it occurs in woods on limestone hills in the cloud forests of primarily the John Crow Mountains from 100–700 m.

Discussion. *Solanum acropterum* is a distinctive species with its strongly winged, completely glabrous pedicels and ellipsoid berries. The inflorescences are borne one to a short shoot, making the flower-bearing portion of the plant look somewhat bushy. In contrast to the other members of the *S. havanense* group, the flowers of *S. acropterum* are white rather than purple. Mature fruits of *S. acropterum* are not known, but appear to be ellipsoid and are likely to be blue-green, purple, or black, as are the fruits of *S. havanense*.

Solanum acropterum is known only from the John Crow Mountains and a few other forest habitats in central Jamaica; due to this restricted distribution on a single island, it is likely to be of conservation concern (Yoder et al., in prep.) and was listed in 1997 as Rare (Walter & Gillett, 1998). This species can be assigned a preliminary conservation status of Vulnerable (VU) (IUCN, 2001), but there is a reasonable basis to consider *S. acropterum* as Endangered (EN) due to paucity of collections and its status as a single island endemic (Yoder, 2006).

Grisebach (1862) cited two collections in his original description of *Solanum acropterum*, *Waters s.n.* and *Wilson s.n.*, both at K. The Waters sheet (K 000005250) has been chosen as the lectotype because it has both flowers and fruit (Fig. 1). Neither sheet has specific collecting locality information.

Additional specimens examined. JAMAICA. s. loc., 1844, *Purdie s.n.* (K [2]); *Wilson s.n.* (K). **Hanover Parish:** Dolphin Head & vic., *Britton 2294* (K, NY), *Harris 10298* (BM, F, NY, US). **Portland Parish:** E slopes, John Crow Mtns., *Britton 4137* (NY). **Saint Ann Parish:** Union Hill, near Moneague, *Britton & Hollick 2807* (NY, US); Stirling Castle Forest Reserve, vic. Alumina Jamaica tramline, *Howard 15024* (A). **Saint Thomas Parish:** E slopes of S end of John Crow Mtns., *Harris & Britton 10723* (F, K, NY, US), *Harris 10752* (F, K, NY, US); Big Level Distr., SE end of the John Crow Mtns., *Proctor 11802* (US), *Stearn 499* (A, BM [4], K [2], MO), *Webster & Proctor 5546* (BM, G, NY, US). **Trelawny Parish:** Paynes Patent, 2 mi. WNW of Quick Step, 9 Mar. 1949, *Lewis s.n.* (US); Windsor, *Miller 1490* (US).

2. Solanum conocarpum Dunal in Poir., Encycl. Suppl. 3: 748. 1814. TYPE: U.S. Virgin Islands. St. John: Coral Bay, *L. C. Richard s.n.* (holotype, P!; isotype, F 976721 [fragm.]!). Figure 2A.

Figure 1. Lectotype specimen of *Solanum acropterum* (*Waters s.n.* [K]).

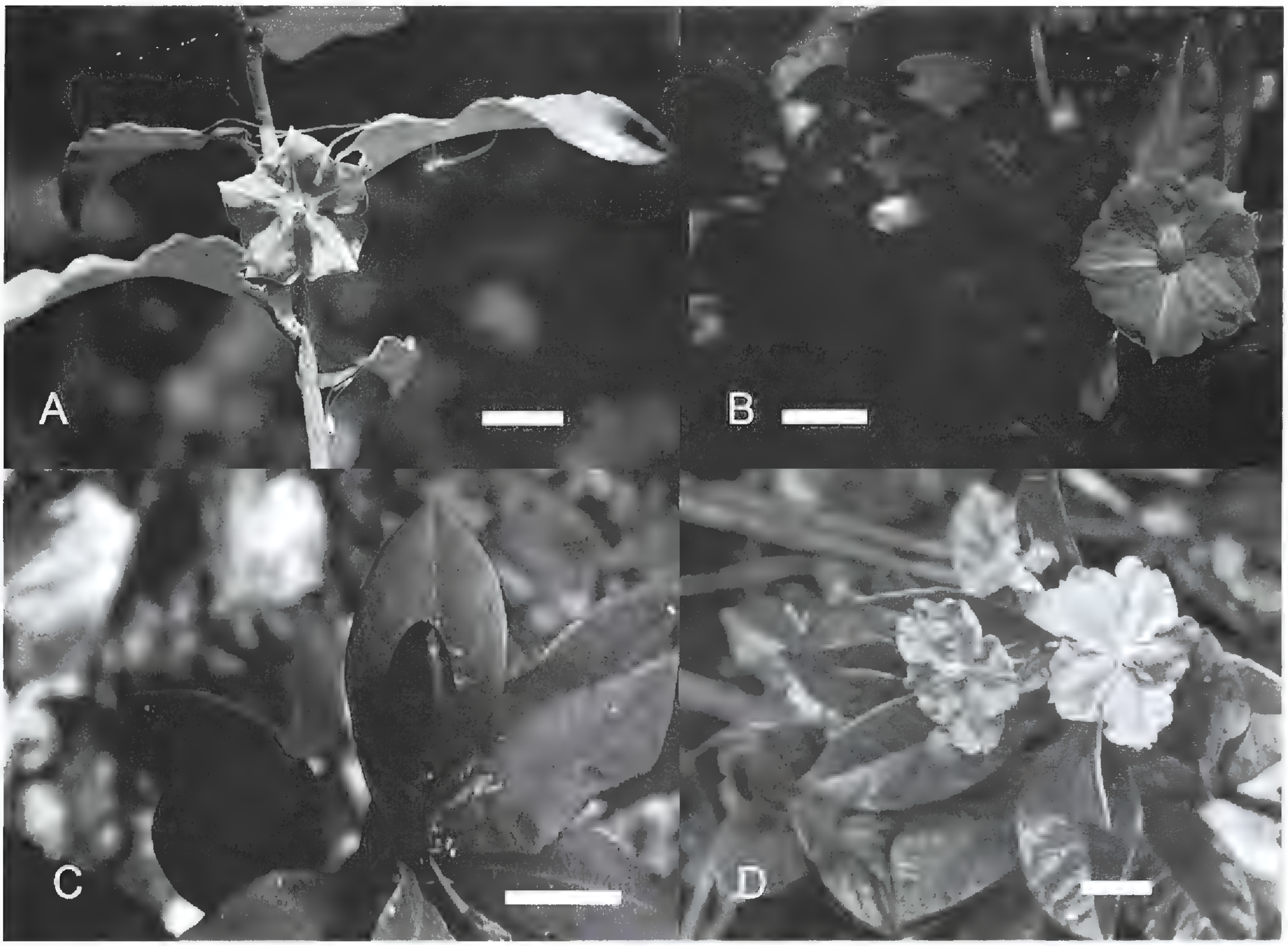

Figure 2. Flowers and fruit of members of the *Solanum havanense* species group. —A. *S. conocarpum* flower (photo, P. Acevedo). —B. *S. havanense* flower (photo, C. D. Adams). —C. *S. havanense* fruit (photo, C. D. Adams). —D. *S. troyanum* flowers (photo, C. D. Adams). Scale bars = 1 cm.

Shrubs to 2 m; stems ± stout, erect, pale grayish white to brown, glabrescent; new growth densely pubescent with simple uniseriate trichomes ca. 0.5 mm, soon glabrescent; bark white, exfoliating; sympodial units plurifoliate. Leaves 3–6 × 1.5–2.5 cm, elliptic, glabrous on both surfaces, the base acute, the margins entire, the apex acute to acuminate; primary veins 7 to 8 pairs, sometimes drying yellowish green; petiole 0.5–0.8 cm, glabrous. Inflorescences leaf-opposed, terminal on short axillary shoots, 0.4–0.6 cm, simple, with 1 to 3 flowers, densely pubescent with simple, uniseriate trichomes to 0.5 mm, the peduncle absent to 0.3 cm; pedicels 2–4 mm, stout, ca. 1 mm diam. at base and apex, erect at anthesis, glabrous, articulated at the base; pedicel scars closely spaced, somewhat overlapping; buds globose, the corolla ca. 1/2 to completely exserted. Flowers heterostylous; calyx tube 1–2 mm, broadly cup-shaped, glabrous, the lobes 1.5–2 mm, deltate, glabrous with an apical tuft of uniseriate trichomes; corolla 1.5–2 cm diam., bright purple or violet, lobed ca. 1/4 to 1/3 of the way to the base, the lobes ca. 2 × 1 mm, broadly deltate, planar at anthesis, densely papillose-pubescent at the tips; filaments glabrous, the free portion 1–15 mm, the filament tube less than 0.5 mm or absent; anthers 2–2.5 mm, poricidal at the tips, the pores with thickened edges, slightly lengthening to slits with age; ovary long-conical, the style 6–8 mm in long-styled flowers, less than 2 mm in short-styled flowers, glabrous, the stigma broadly capitate, the surface minutely papillose. Fruit a conical berry, 3–5 × 2–3.5 cm, hard and green at maturity, the pericarp woody, the apex sharply pointed; fruiting pedicel 2–3 cm, ca. 5 mm diam. at the base, woody and pendent from the weight of the berry; seeds not seen. Chromosome number not known.

Distribution. Endemic to the island of St. John in the U.S. Virgin Islands (and perhaps also St. Thomas and Virgin Gorda, fide Acevedo-Rodríguez, 1996), growing in seasonally dry, coastal habitat, in forest understory or in open areas from 0–500 m.

Common name. "Marron bacoba" (Dunal, 1852).

Discussion. *Solanum conocarpum* is quite similar in vegetative morphology to *S. havanense*, and it inhabits the same sorts of coastal habitats. The two species do not appear to be sympatric, and *S.*

havanense is found on Cuba, Jamaica, and the Cayman Islands but is not found in the Virgin Islands. The flowers of *S. conocarpum* are more stellate in outline than those of *S. havanense*, and usually have a distinct white eye (see Fig. 2A, B). The fruits of the two species are completely different; *S. conocarpum* has very large, conical berries that are green at maturity and are borne on woody, pendent pedicels, while the berries of *S. havanense* are smaller, ellipsoid, and dark purplish blue at maturity and are borne on erect pedicels (Fig. 2C).

Solanum conocarpum was proposed for listing as endangered under the U.S. Fish and Wildlife Endangered Species Act in 1998, but in 2006 the decision was made that the listing was not warranted due to lack of information about this species (<http://www.fws.gov/southeast/news/2006/r06-016.html>). Based on the paucity of collections and the current population data, this species can be assigned a preliminary conservation status of Critically Endangered (Yoder, 2006). The record of *S. conocarpum* from Puerto Rico cited in the 1997 IUCN Red List (Walter & Gillett, 1998, based on a record in The Nature Conservancy's 1996 National Heritage Database, not available online) is likely to be an error. I have seen no authentic material of *S. conocarpum* from Puerto Rico.

Acevedo-Rodríguez (1996) knew of *Solanum conocarpum* only from a single plant on St. John but suggested it might also occur on St. Thomas and Virgin Gorda. I have seen no material from either island, although Acevedo-Rodríguez cites a sterile specimen (*Little 23836*) from Virgin Gorda that I have not examined. Forbes et al. (pers. comm., 2005) have found fewer than 200 individuals of *S. conocarpum* in the wild and have suggested that the species is self-incompatible, since plants do not set fruit when grown in isolation. *Solanum conocarpum* has suffered severe loss of genetic diversity (Stanford et al., 2002; A. M. Stanford, pers. comm.), indicating that this species is a high priority for listing at both a global and national level.

Additional specimens examined. U.S. VIRGIN ISLANDS. **St. John:** Reef Bay Quarter, along trail from Lameshur to Reef Bay ruins, *Acevedo et al. 5437* (K, MO).

3. Solanum havanense Jacq., Enum. Syst. Pl. 15. 1760. TYPE: Jamaica. Tab. 35, Jacquin, 1763 (neotype, designated here, tab. 35 in Jacquin, 1763!). Figures 2B, C, 3.

Solanum hookerianum A. Spreng., Tent. Suppl. 9. 1828, replacement name for *Solanum coriaceum* Hook., 1827. Replaced synonym: *Solanum coriaceum* Hook., Bot. Mag. 54: 2708. 1827. *Diamonon coriaceum* (Hook.) Raf., Fl. Tellur. 2: 76. 1836 [1837]. *Solanum byrsinum* Voigt, Hort. Suburb. Calcutt. 511. 1845, nom. illeg., superfl. TYPE: Plate 2708, based on a plant cultivated in England, the seeds said to be sent by R. Barclay from Mexico (lectotype, designated here, plate 2708 in Hooker, 1827!).

Solanum myrtifolium Lodd., Bot. Cab. 25: pl. 1431. 1828. TYPE: Plate 1431, based on a plant cultivated in London (lectotype, designated here, plate 1431 in Loddiges, 1828!).

Solanum havanense Vell., Fl. Flumin. 82. 1829, nom. illeg. TYPE: Icon. Tab. 94, Brazil. Rio de Janeiro: Pharmocopolis (lectotype, designated here, tab. 94 in Vellozo, 1827 [1831]!).

Solanum vacciniifolium Dunal, Prodr. (DC.) 13(1): 160. 1852. *Solanum havanense* Jacq. var. *vacciniifolium* (Dunal) Griseb., Cat. Pl. Cub. 189. 1866. TYPE: Cuba. Havana, *de la Sagra 294* (holotype, G-DC!, F neg. 33944!, IDC microfiche 800-61.2075:I.2!).

Shrubs to 3 m; stems erect, glabrescent; bark dark or pale gray; new growth densely pubescent with minute appressed, simple or occasionally dendritic uniseriate trichomes to 0.5 mm, soon glabrescent; sympodial units plurifoliate. Leaves (1.5)2–9 × (0.8)1.1–4.5 cm, elliptic, thick and coriaceous, glabrous and shiny adaxially, sparsely pubescent abaxially with minute simple and dendritic uniseriate trichomes less than 0.5 mm along the main veins and with scattered longer trichomes to 1 mm, the base attenuate, the margins entire and revolute, the apex acute or sometimes rounded; primary veins 5 to 6 pairs, drying reddish orange; petioles 0.2–1 cm, pubescent with uniseriate trichomes like the stems. Inflorescences leaf-opposed, internodal or sometimes on short shoots, 0.4–1.3 cm, with 3 to 6 flowers, simple, densely pubescent with simple uniseriate trichomes to 0.5 mm, the peduncle 0.2–0.5 cm; pedicels 0.6–1 cm, ± fleshy, ca. 1 mm diam. at base and apex, erect at anthesis, sparsely pubescent with simple uniseriate trichomes like the inflorescence, articulated at the base; pedicel scars ca. 1–2 mm apart, articulated at the base or with a small peg remaining after abscission; buds globose, the corolla included until just before anthesis. Flowers apparently all perfect; calyx tube 1 2 mm, cup-shaped, the lobes 3–5 mm, lanceolate, tearing at anthesis along the sinuses, sparely pubescent with a tuft of simple uniseriate trichomes at the apex; corolla 2–3 cm diam., violet, fading to pale violet or white, lobed ca. 1/4 of the way to the base, the lobes splitting as anthesis proceeds, the lobes 0.5–1 cm, deltate to ovate, planar at anthesis, densely white papillose at the tips; filaments with the free portion 1–1.5 mm, the filament tube less than 0.5 mm or absent; anthers 3.5–4 × 1.5–2.5 mm, poricidal at the tips, the pore not lengthening; ovary glabrous, somewhat conical, the style 7.5–9 mm, glabrous, the stigma capitate, the surface minutely papillate. Fruit an ovoid to ellipsoid berry, 1–1.2 × 0.9–1.2 cm, dark blue-purple or black at maturity, soft and fleshy, the pericarp thin, the apex

Figure 3. Neotype of *Solanum havanense* (Jacquin, 1763: tab. 35). Image reproduced with permission of Botany Library, The Natural History Museum.

rounded; fruiting pedicels 1–1.2 cm, woody and erect, ca. 1 mm diam. at the base; seeds ca. 7 × 3 mm, ovoid, reddish brown, the surfaces minutely pitted, the testal cell walls straight. Chromosome number not known.

Distribution. In woods on limestone from 0–100 m, on Cuba, Jamaica, and the Cayman Islands.

Common name. Dominican Republic: "roblina" (*Jimenez 1907*).

Discussion. *Solanum havanense* is a striking plant, with its large purple flowers and bright purplish blue to black fruits (Fig. 2B, C). It was brought into cultivation in Europe and appears to have been spread widely amongst botanic gardens (see synonyms), with some loss of information as to its original provenance. The flowers of *S. havanense* expand through anthesis, with the corolla lobes becoming both larger and more divided with time (see Fig. 2B; and shown clearly in Hooker, 1827). The corolla color also fades from bright to pale purple, and the lobe orientation becomes more planar as the flower ages (Fig. 2B).

Solanum havanense is relatively common and widespread in the Greater Antilles, occurring on Cuba, Jamaica, and the Cayman Islands (a single collection, possibly cultivated), but apparently absent from Puerto Rico and Hispaniola; the single specimen from the Dominican Republic (*Jiménez 1907*) is most probably cultivated. Specimen data indicate that it is relatively common, although its habitat is the coastal fringe, making it possibly at risk from coastal development. It has been assigned a preliminary conservation status of Near Threatened (NT) and standard monitoring is suggested (Yoder, 2006).

No specimen of *Solanum havanense* attributable to Jacquin has been found, despite intensive searches in many herbaria (principally BM, LINN, and W; see Stafleu & Cowan, 1979) where such a sheet, if it had ever existed, might have been preserved. No original material was cited in the protologue (Jacquin, 1760), so any type selected must be a neotype. The illustration of *S. havanense* in Jacquin's first illustrated edition of the *Selectarum Stirpium Americanarum Historia* (Jacquin, 1763), a copper engraving taken from original drawings done by Jacquin in the field (see Wiltshear, 1913), is very accurate, and I have chosen it here as the neotype of the species (Fig. 3). The much more detailed description in Jacquin (1763) mentions the distinctive blue fruit of *S. havanense*, and the plate in the hand-painted color edition of *Selectarum Stirpium Historia Iconibus* (Jacquin, 1780) clearly shows this. Similarly, specimens of neither *S. coriaceum* nor *S. myrtifolium* have been found. A specimen of *S. havanense* labeled as "Solanum coriaceum Hook. Bot. Mag." at Kew (K 00005238) is not clearly associated with the description and does not have the distinctive calyx morphology depicted in the plate. These names too are lectotypified using the original illustrations (Figs. 4, 5). Vellozo's (1825) description of *S. havanense* may be a re-description of Jacquin's plant, but no reference to Jacquin is made in the protologue or accompanying material. I have lectotypfied it using the illustrations published in 1831, as no herbarium specimens are extant from Vellozo's work (Carauta, 1973). *Solanum hookerianum* (Sprengel, 1828: 9) and *S. byrsinum* (Voigt, 1845: 511) were both proposed as replacement names for *S. coriaceum* Hook., which is homonymic with *S. coriaceum* Dunal, a spiny vine from northern South America.

Additional specimens examined. CAYMAN ISLANDS. **Grand Cayman:** 3/4 mi. N of Half Moon Bay, *Brunt 2200* (BM). CUBA. s. loc., *Anon. s.n.* (MO); San Antonio, *Hitchcock s.n.* (F); s. loc., *Mèrat de Vaumartoise s.n.* (W); Lemonal, May 1823, *Poeppig s.n.* (W); s. loc., *Poeppig s.n.* (W); s. loc., *de la Sagra s.n.* (W); s. loc., *Wright 3025* (BM, G, G-DC, GH, K, MO, US, W); Santa Clara, vic. of Soledad, along creek which crosses trail to Guaos, *Howard 5740* (GH, JBSD, US). **La Habana:** Cojímar, *Alain 2394* (US), *Alain, Bro. 2396* (GH); Sierra del Grillo, Madruga, *Alain 5280* (GH); Havana, *Anon. s.n.* (W); La Playa de Marianao, *Curtiss 716* (A, BM, F, G, K, MO, US); Loma Esperon, *Ekman 10555* (F); Sierra de Anafe, *León 7135* (GH); Tamory Mtes, *Rugel 94* (BM). **Matanzas:** Matanzas, *Rugel, F. 144* (BM [2], K). **Pinar del Rio:** base of Sierra de Jíbaro, El Ancón, Viñales, *Alain 4292* (GH, US); Bay of Mariel, *Britton & Earle 7609* (US); Mariel Tinaja, *Ekman 12876* (A, G); Loma Harenales, S of Sumidero, *Schafer 3795* (A, US); Loma de Esperon, on the NW outskirts of Caimito, near border of Habana province, *Webster 3700* (GH, US); Sierra de Anafe, *Wilson 11305* (US). **Santa Clara [Cienfuegos]:** Cienfuegos, Cieneguita N, *Combs 114* (F [2], GH, K, MO); Guabairo, Soledad, Cienfuegos, *Jack 7317* (A, F, US); Blanco's Woods, near the E shore of Cienfuegos Bay, SW of Soledad, *Webster et al. 51* (A). **Villa Clara:** Dolores near Soledad Central, *Howard 6590* (A [2]). DOMINICAN REPUBLIC. **Santiago:** Ciudad de Santiago, *Jiménez 1907* (US). JAMAICA. Hector's River, *Harris & Britton 10718* (K, NY, US); s. loc., *MacFadyen s.n.* (K [2]), *Mar 1666* (K), *Wilson s.n.* (GH), *Wilson s.n.* (K [2]), *Wright s.n.* (BM, K). **Manchester Parish:** Gut River, *Adams 6333* (BM); 1 mi. ESE of God's Well, N side of Round Hill, *Stearn 634* (BM). **Portland:** Priestman's Bay, *Bailey 731* (US); vic. of Port Antonio, mouth of Priestman's River, *Britton 891* (F, NY); Long Bay, *Britton 4213* (NY); Priestman's River, *Proctor 6104* (US); near Rural Hill, *Proctor 31565* (MO); Turtle Cove, near Bennett Point, about 2 mi. due E of Port Antonio, *Stearn 537* (BM); near mouth of Priestman's River, *Yuncker 18423* (NY). **Saint Andrew Parish:** vic. of Kingston, Ferry River, *Britton 401* (F, NY), *Britton 2831* (NY); Ferry Pen, *Campbell 6175* (F); hill behind the Quarry at the Ferry, *Harris 10036* (BM, F, NY, US), *Harris 10036* (K); Hector's River, 9 Mar. 1909, *Harris & Britton 10718* (F); Ferry Pen, *Herb. Bot. Dept. Jamaica 6175* (NY), *Herb. Bot. Dept. Jamaica 6248* (GH); W side of Ferry Hill, 3/4 mi. up Old Ferry Rd., near Fresh River, *Stearn 821* (BM); Ferry Hill, Ferry Pen, *Webster et al. 8076* (BM, G [2]). **Saint Ann Parish:** Fort Point, Sep. 1955, *Asprey 2353* (NY); Queens Hwy., 13 Dec. 1970, *Plowman*

Figure 4. Lectotype of *Solanum coriaceum* (Hooker, 1827: tab. 2708). Image reproduced with permission of Botany Library, The Natural History Museum.

Figure 5. Lectotype of *Solanum myrtifolium* (Loddiges, 1828: pl. 1431). Image reproduced with permission of Botany Library, The Natural History Museum.

2986 (GH); along Queen's Hwy., about 2 mi. E of Rio Bueno, *Stearn 140* (BM, K). **Saint Elizabeth Parish:** Pepper, *Britton 1073* (NY); Font Hill, near Black River, Luana Point, *Britton 1511* (NY); Kaiser mine area S of Gutters, *Howard & Proctor 13765* (A, NY); Pepper, *Miller 1358* (US); Fort Charles, Feb. 1936, *Sangster s.n.* (BM); vic. of Merriman's Point, *Stearn 865* (BM). **Saint James Parish:** Orange River valley, 1/2 mi. SE of Sign, *Proctor 16625* (BM). **Saint Thomas Parish:** Great Morass, *Adams 11833* (MO); Holland Bay, *Purdie 135* (K). **Trelawny Parish:** near Rio Bueno along coast rd. to Duncane, *Plowman 3241* (A, K, US); White Bay, 4 mi. due E of Falmouth, *Proctor 23668* (BM); ca. 2 mi. W of Rio Bueno Harbour, *Stearn 319* (BM). **Westmoreland Parish:** Negril & vic., *Britton & Hollick 2058, 2063* (NY); Negril, *Harris 10231* (BM, F, K, NY, US), *Harris 10237* (K, NY).

4. Solanum troyanum Urb., Symb. Antill. 5: 487. 1908. TYPE: Jamaica. Trelawny Parish: Mt. Diablo, 29 Aug. 1905, *W. Harris 9000* (lectotype, designated here, BM 000849163!; isotypes, K 000005227!, NY 00111387!). Figures 2D, 6.

Slender shrubs to small trees, to 4 m; stems ± stout, erect, glabrous; bark very dark on new shoots, white on older stems, peeling and exfoliating in irregular patches; new growth completely glabrous, drying black; sympodial units plurifoliate. Leaves 4.5–16.5 × 1.5–6 cm, elliptic, thick and somewhat fleshy, shiny adaxially, matte abaxially, completely glabrous, drying dark, the base attenuate, the margins entire, slightly revolute, the apex acute; primary veins 6 to 9 pairs, drying reddish green; petioles 1–1.5 cm, glabrous. Inflorescences leaf-opposed on short terminal leafy shoots, 3–8 cm, simple or occasionally once-branched, with 3 to 10 flowers, glabrous, the peduncle 1.5–3 cm; pedicels 2–2.5 cm, stout and fleshy, 1–1.5 mm diam. at the base and the apex, ± erect at anthesis, articulated just above the base; pedicel scars 5–8 mm apart, the pedicels flush with the rachis or leaving a peg 0.5–1 mm; buds globose to ellipsoid, strongly exserted from the calyx tube. Flowers apparently all perfect; calyx tube 3–4 mm, broadly cup-shaped, glabrous, the lobes 5–6 mm, deltate and overlapping, minutely papillate on the tips and margins; corolla 3.5–6 cm diam., violet, intensely fragrant, lobed 1/4–1/2 of the way to the base, the lobes 0.5–2 cm, deltate and emarginate, enlarging with age as corolla expands, planar to somewhat campanulate at anthesis, densely white papillate at the tips; filaments with the free portion 2.5–3 mm, the filament tube less than 0.5 mm, glabrous; anthers 6–7 × 2.5–3 mm, poricidal at the tips, the pores not lengthening to slits with age; ovary conical, glabrous, the style ca. 15 mm, straight, glabrous, the stigma minutely capitate, the surface minutely papillate. Fruit a globose berry, ca. 1–1.5 cm diam. (immature), dark bluish green, the pericarp thin; fruiting pedicels 3–4 cm, 1.5–2 mm diam. at the base, woody, erect; seeds 4–5 × 2.5–3 mm, ovoid-reniform, pale tan, the surfaces minutely pitted, the testal cell margins sinuate. Chromosome number not known.

Distribution. Endemic to Jamaica, in moist crevices and hilltops on limestone, from 500–1000 m.

Discussion. Solanum troyanum is a spectacular plant with its large, purple flowers to 6 cm diam. and shiny dark green leaves. Like those of *S. havanense*, the flowers become larger through anthesis. *Solanum troyanum* is very similar to *S. havanense* but, in addition to having larger flowers, is glabrous on all vegetative parts, including the very young growth. *Solanum havanense* occurs along the coastal fringe on limestone, while *S. troyanum* is a mountain plant, growing in moist forests on slopes.

Solanum troyanum is assigned a preliminary conservation status (IUCN, 2001) of Vulnerable (VU) based on its narrow geographical range (< 20,000 km^2) and the length of time since the last specimen was collected (1976: Yoder, 2006), but its status as a single island endemic may warrant an assessment of Endangered (EN). Re-collection of *S. troyanum* is a high priority, and given its large, showy flowers, it is a potential candidate for ex situ conservation and propagation as an ornamental.

Three syntypes were cited by Urban in the original description (Urban, 1908): *Harris 8530, 8681*, and *9000*. The gathering *Harris 9000* from Mt. Diablo was chosen as the lectotype (BM, see Fig. 6) as it represents more complete flowering and fruiting material, and is distributed in three herbaria.

Additional specimens examined. JAMAICA. **Clarendon Parish:** vic. of Quaco Rock, vic. Ritchies, *Proctor 33808* (BM, MO). **Manchester Parish:** Banana Ground, *Adams 11230* (BM, MO); Christiana, 1/2 mi. NW of Christiana, *Howard & Proctor 14365* (A, BM); Coleyville, Gourie Forest, ca. 1 mi. due SW of Coleyville, *Proctor 31768* (F, MO). **Saint Ann Parish:** Douglas Castle Distr., *Proctor 26410* (BM, GH). **Saint Catherine Parish:** vic. of Hollymount, Mt. Diablo, *Maxon 2206* (NY, US [2]). **Saint Elizabeth Parish:** Mulgrave, along rd. betw. Mulgrave & Elderslie, *Proctor 20480* (BM). **Saint James Parish:** Sweetwater, 1 mi. S of Sweetwater, *Proctor 7980* (US). **Trelawny Parish:** Troy, 5.5 mi. NW of Troy, *Adams 12827* (BM); Troy, Cockpit country, *Britton 591* (NY); near Troy, *Harris 8530* (A, BM, NY), *Harris 8681* (NY [2]), *Perkins 1375* (GH); Island View Hill, Wilson Valley Distr., 1.5 mi. N of Warsop, *Plowman 3256* (A, K, US); Troy, *Proctor 9911* (NY); Island View Hill, Wilson Valley distr., 1.5 mi. N of Warsop, *Proctor 21339* (BM); Miss Laura's Hill, Wilson Valley distr., ca. 1 mi. N of Warsop, *Proctor 24845* (A, BM, NY, US); Burnt Hill, overhanging Donkey Trail, 1/2 mi. N of Burnt Hill intersection, *Read 1918* (BM, GH, US); Troy, Bartley's Run, near Troy (ca. 3 mi. NNW), *Stearn 922* (BM [2]); Island View Hill, N of Warsop, *Webster 13669* (BM); Burnt Hill, *Whitefoord 1399* (BM, F, MO).

Figure 6. Lectotype of *Solanum troyanum* (*Harris 9000* [BM]).

ADDITIONS TO THE *SOLANUM PSEUDOCAPSICUM* SPECIES GROUP

Small shrubs, often with woody tap roots; young stems and leaves glabrous or densely pubescent, the trichomes either simple and uniseriate or dendritic; sympodial units difoliate, geminate, the minor leaves similar in shape to the major leaves, but usually smaller. Leaves simple, lanceolate to elliptic, glabrous to densely pubescent, the trichomes like those of the stems. Inflorescences opposite the leaves, usually simple but in some species many times branched. Flowers white, the calyx lobes deltate to leafy and relatively large, the corolla lobes and sepals reflexed at anthesis. Fruit an orange or red berry, soft and juicy at maturity; fruiting pedicels erect, thickened and woody; seeds flattened-reniform, pale yellowish brown, the surfaces pitted.

Distribution. Species of the group are widespread in dry habitats, from Mexico to Uruguay.

5. Solanum argentinum Bitter & Lillo, Repert. Spec. Nov. Regni Veg. 12: 547. 1913. TYPE: Argentina. Córdoba: prope Córdoba, *T. Stuckert 15164* (lectotype, designated here, CORD!; isotypes, G [2]!, LIL not seen). Figure 7.

Solanum argentinum Bitter & Lillo var. *chroniotrichum* Bitter, Repert. Spec. Nov. Regni Veg. 12: 549. 1913. *Solanum chroniotrichum* (Bitter) C. V. Morton, Revis. Argentine Sp. Solanum 183. 1976. TYPE: Argentina. Jujuy: s. loc., *T. Stuckert 21337* (neotype, designated by Morton, 1976: 183, CORD!; isotype, G!).

Shrubs (clonal?) to 2 m; stems erect, ca. 0.5 cm diam., sparsely to densely pubescent with uniseriate dendritic trichomes 0.25–1 mm, longer trichomes found in more densely pubescent populations, pubescence extremely variable and correlated over the entire plant; bark red or dark brown, very shiny; new growth sparsely dendritic pubescent like the stems; sympodial units (2)3-foliate, only occasionally geminate. Leaves (2–)3.5–15 × (0.6–)1–5.7 cm, elliptic to narrowly elliptic, adaxially glabrous to sparsely pubescent with uniseriate simple or dendritic trichomes 0.5–1 mm on the veins and lamina, abaxially glabrous to densely pubescent with uniseriate dendritic trichomes 0.5–1 mm over entire surface, the base attenuate, the margins entire, the apex acute to acuminate; primary veins 10 to 12 pairs, often drying yellowish green; petioles 0.3–1.2 cm, glabrous to densely dendritic pubescent. Inflorescences internodal or terminal, 2.5–10 cm, many times (to 6×) branched, with 50 to 200 flowers, sparsely to densely pubescent with dendritic trichomes, the pubescence less dense apically, the peduncle 2–4 cm; pedicels 0.5–0.6 cm, less than 0.5 mm diam. at the base and apex, nodding at anthesis, sparsely dendritic pubescent; pedicel scars closely packed distally, to 3 mm apart toward the base of the inflorescence, plane with the rachis; buds globose, the corolla exserted approximately halfway. Flowers all perfect; calyx tube ca. 1 mm, conical, the calyx lobes 1–1.5 mm, narrowly triangular, sparsely dendritic pubescent abaxially; corolla 0.8–1 cm diam., white, lobed ca. 3/4 of the way to the base, the lobes 0.5–0.8 cm, deltate, planar to slightly reflexed at anthesis, glabrous to densely papillate toward the apex abaxially; filaments with the free portion 1–1.2 mm, the tube essentially absent, glabrous; anthers 2–2.5 × ca. 1 mm, poricidal at the tips, the pores lengthening to slits with age; ovary conical, glabrous, the style 4–5 mm, straight, glabrous, the stigma capitate, the surface minutely papillate. Fruit a globose berry, 0.5–0.6 cm diam., bright orange or yellowish orange at maturity, the pericarp thin, the pulp sticky; fruiting pedicels 0.7–0.9 cm, ca. 2 mm diam. at the base, swollen just below the berry, ca. 1 mm diam. at the apex, woody and erect; seeds 6 to 8 per berry, 3–3.5 × 2–2.5 mm, flattened-reniform, pale yellowish tan, the surfaces minutely pitted. Chromosome number $n = 12$ (Moscone, 1992).

Distribution. Bolivia, Argentina, and Paraguay, primarily a species of Chaco habitats, but in many secondary habitats in a wide range of elevations from 100–3000 m.

Common names. Argentina. "hedionillo del monte" (*Lorentz & Hieronymus s.n.*); Salta: "cabia yuyu" (*Cozzo 77*); Santiago del Estero: "afata" (*Gramaja 16*); Tucumán: "runa-yuyu" (*Stuckert 18156*). Bolivia. Santa Cruz: "amarguillo negro," "yacurembiú" (*Vargas 1052*). Paraguay. Presidente Hayes: "cabara yuyo" (*Soria 364*).

Discussion. I excluded *Solanum argentinum* from section *Geminata* based on its complex, branched inflorescence, and thought it was possibly more closely related to members of *Solanum* sect. *Holophylla* s. str. such as *S. aligerum* Schltdl. (Knapp, 2002a). Analyses of DNA sequences (Bohs, 2005; Weese & Bohs, 2007) show that *S. argentinum* is firmly embedded in the Geminata clade, and that it is apparently close to *S. pseudocapsicum* L. and its relatives. Closer analysis of inflorescence morphology revealed that *S. argentinum* lacks the distinctive platform pedicel base morphology of *S. aligerum* and relatives (see Knapp, 1989), and that the inflorescence, despite its highly branched character, is similar to those elsewhere in the Geminata clade. *Solanum argentinum* shares with other members of the *S. pseudocapsicum* species group (sensu Knapp, 2002a) brightly colored fruit with red or orange

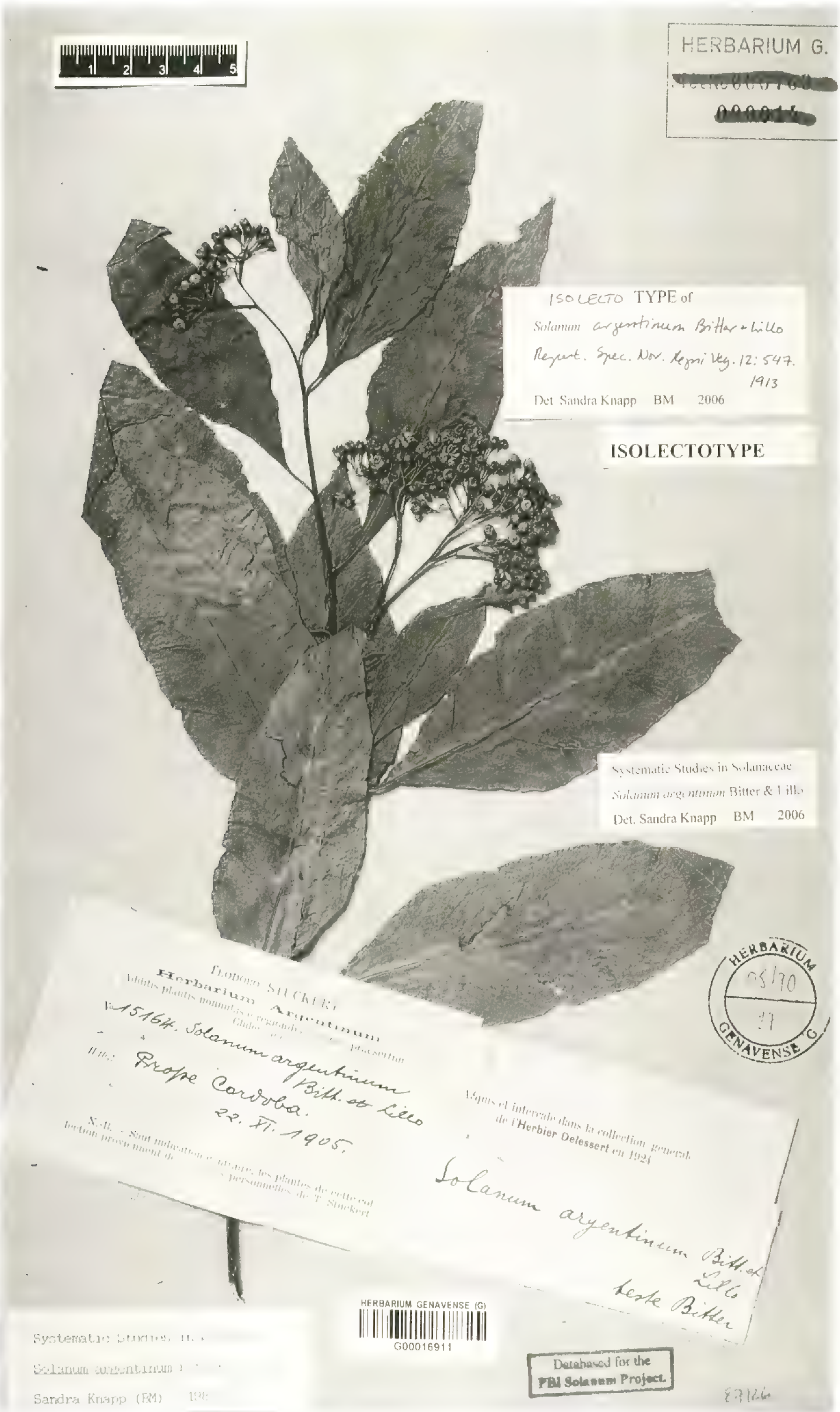

Figure 7. Isolectotype of *Solanum argentinum* (*Stuckert 15164* [G]).

pericarp, yellow, flattened seeds, and pedicels that are nodding in flower and erect in fruit.

Individual plants of *Solanum argentinum* vary enormously in pubescence density, from nearly glabrous to densely pubescent over the entire abaxial leaf surface. This variability in pubescence density led to the description of the two taxa now synonymized under *S. argentinum*. Rigonatto et al. (2005) examined trichome and leaf surface morphology along the range of pubescence densities and found no differences that would lead them to regard them as different taxa, even at the subspecific level. Pubescence density may also vary in response to environmental variables such as aridity or elevation. To test this possibility, I scored mature leaves (four leaves below the growing tip of a shoot) into three classes of pubescence density on 160 geo-referenced specimens with elevation recorded (see Appendix 3) from throughout the species range. Each duplicate of a given collection was scored separately, as some collections were obviously not from the same plant (i.e., they were very different in pubescence density). The scoring was a simple three-point scale: 1 = glabrous, 2 = tufts of trichomes in the vein axils, 3 = pubescence on the entire lamina. The data are displayed as box plots in Figure 8. No effect of latitude, longitude, or altitude was apparent, and the confidence limits of each pubescence class overlapped for each environmental variable (see Fig. 8). Local populations of *S. argentinum* are often composed of large continuous patches of plants of identical pubescence density. My observations of populations in eastern Bolivia (e.g., *Nee et al. 51722*, *51723*) suggest to me that the species may be clonal, or spreading through underground connections. This, however, has not yet been investigated.

Solanum argentinum is a widespread and weedy species. It can be assigned a preliminary conservation status (IUCN, 2001) of Least Concern (LC).

Bitter and Lillo (Bitter, 1913) cited 16 collections in their original description, most of which were listed without herbaria. Those that were identified as being in a particular herbarium were either from Berlin, herbarium Hieronymous (subsequently held at Berlin) or herbarium Stuckert (now at G). Morton (1976) identified potential lectotypes in CORD, but did not lectotypify *Solanum argentinum*, as he had not seen material from Teodoro Stuckert's herbarium in Geneva at the time he was writing his treatment of Argentine *Solanum*. I have chosen *Stuckert 15164* as the lectotype of *S. argentinum*, the specimen in G is annotated in Bitter's hand, and duplicates are in CORD and LIL (fide Morton). I have chosen the lectotype as the specimen in CORD so as to place the type of this species in its country of origin.

Additional specimens examined. ARGENTINA. ca. Tapia, *Baer 110* (G); Río Juramento, 1972, *Morton s.n.* (MO, US); FAQIA, *Rodriguez 21* (US); s. loc., *Stuckert 8896* (G); Capital Federal, Villa Ortúzar, cult. Hort. Bot. Fac. Agr. y Vet, *Weisz BAA5761* (US). **Catamarca:** Desmonte, *Bartlett 19631* (US); Santa Rosa, *Brizuela 166* (W); Dept. La Paz, Aibas, *Brizuela 523* (MO); Dept. La Paz, El Aibal, *Brizuela 744* (G, W); Dept. La Paz, El Lindero, *Brizuela 893* (MO); Dept. La Paz, El Divisadero, *Brizuela 1145* (G); Dept. Ancasti, El Carrizal, *Brizuela 1334* (W); Dept. Paclín, La Merced, *Pierotti s.n.* (US); Dept. Santa Rosa, Lavalle, *Quintero 63* (MO); Dept. Paclín, Superi, *Risso 156* (MO); Dept. Paclín, La Merced, *Risso 239* (W); Dept. Paclín, La Merced, Río Paclín Nte, *Risso 295* (NY). **Chaco:** Avia Terai, *Aguilar 161* (W); Dept. Napalpí, Presidencia Roque Saenz Peña, *Buratovich 446* (G); Dept. Resistencia, Fortin Cardoso, *Malvárez 1335* (W); Dept. Resistencia, Cote Lai, *Malvárez 1401* (MO); Dept. 1 de Mayo, Colonia Benítez, *Schulz 3839* (F); Dept. Tapenaga, Enrique Urién, *Schulz 8170* (US); Dept. Maipú, Ruta 95, ruinas Km 75 al N de Saenz Peña, *Schulz 15635* (F); Colonia Benítez, *Schulz 15825* (F), *Schulz 17038* (F). **Córdoba:** Gutenberg, Ferrocarril Central de Argentina, *Bartlett 19888* (US); Dept. Ischilin, Quilino, *Meyer 13127* (W); Dept. Río Seco, along Hwy. 9, *Nee 50736* (NY); Villa Maria, *Stuckert 16820* (G); Cruz del Eje, *Stuckert 17237* (G); Dept. Ischilin, Quilino, *Villafañe 74* (US). **Corrientes:** s. loc., *Burkart 2585* (G), *Pedersen 2585* (MO, US); Corrientes, near Facultad de Agronomia, *Plowman 2729* (F, US). **Formosa:** Río Pilcomayo, barrancas a la altura del Campamento Central, *Cordini 67* (US); comun Ric. 83, *Jorgensen 2622* (MO, US); Dept. Pilagás, SE a 3 km de Espinillo, *Morel 7268* (US); Dept. Pilagás, Espinillo, *Morel 7350* (W); Dept. Bermejo, Nuevo Pilcomayo, *Pierotti 4012* (F, NY, US); Dept. Bermejo, Laguna Yema, alrededores de Laguna Yema, *Vanni et al. 4334* (NY). **Jujuy:** alrededores de la ciudad, *Araque M. & Barkley 19Ar482* (W); La Esperanza Estate, San Pedro de Jujuy, *Bartlett 20321* (US); Sierra de Zapla, *Burkart 12017* (MO); Dept. Santa Bárbara, entre El Sauzal y Palma Sola, *Cabrera et al. 31020* (F); Dept. Santa Bárbara, Aguas Calientes, *Cabrera et al. 31427* (MO); Dept. El Carmen, Perico, Río Perico, *Krapovickas & Cristóbal 17526* (US); Dept. El Carmen, Las Lajitas, *Krapovickas et al. 56552* (MO); Perico, *Lillo 9837* (US); Dept. Valle Grande, ca. 0.5 km from Huacanqui on rd. from San Francisco to Valle Grande, ca. 7.5 km N of San Francisco, *Nee & Bohs 50828* (NY). **Salta:** Joaquín V. González, *Aguilar 304* (BM); Dept. Anta, Joaquín V. Gonzalez, *Alvarez 226* (US); vic. of the dam (El Dique), Coronel Moldes, *Bartlett 19683* (US); Dept. Orán, Ballivián–Agua Linda, *Borsini 744* (W); Dept. Orán, Puesto de Agua Tranquila, *Borsini 6721* (W); Dept. Cafayate, ca. 5 km W de Cafayate, Río Colorado, *Charpin & Novara 20717* (G, US); Dept. La Viña, ruta 68, Km 110, ca. El Hongo, *Charpin & Lazare 23928* (G [2]); Dept. La Viña, Salamancha, bords de la ruta 48, *Charpin 25819* (G [2]); Dept. Orán, Embarcación, *Comision para la Medicion de un Arco de Meridiano 896* (F); Orán, alrededores, *Cozzo 77* (US); Dept. Capital, Km 34 al E de Salta, *Descole 1494* (W); Dept. Cafayate, Cafayate, *Hayward 2078* (US), 19 Oct. 1948, *Fries s.n.* (MO); s. loc., *Herb. Hoehne 38942* (US); Dept. Metán, Río Piedra, *Huidobro 536* (US); Río Pasaje Juramento, *Huidobro 617* (US); Dept. La Viña, Coronel Moldes, *Hunziker 1064da* (US); Dept. Rosario de la Frontera, Talayaco, 28 km E de Rosario de la Frontera, ruta 34, *Krapovickas et al. 28017* (MO); Dept. Rosario de Lerma, Cerrillos, *Krapovickas et al. 28397* (MO); Dept. San Martín, 16 km W de Gral. Ballivián, orilla de arroyo afluente del Río Seco, *Krapovickas & Schinini 30909* (F, MO); Dept. San Martín, Campo Durán,

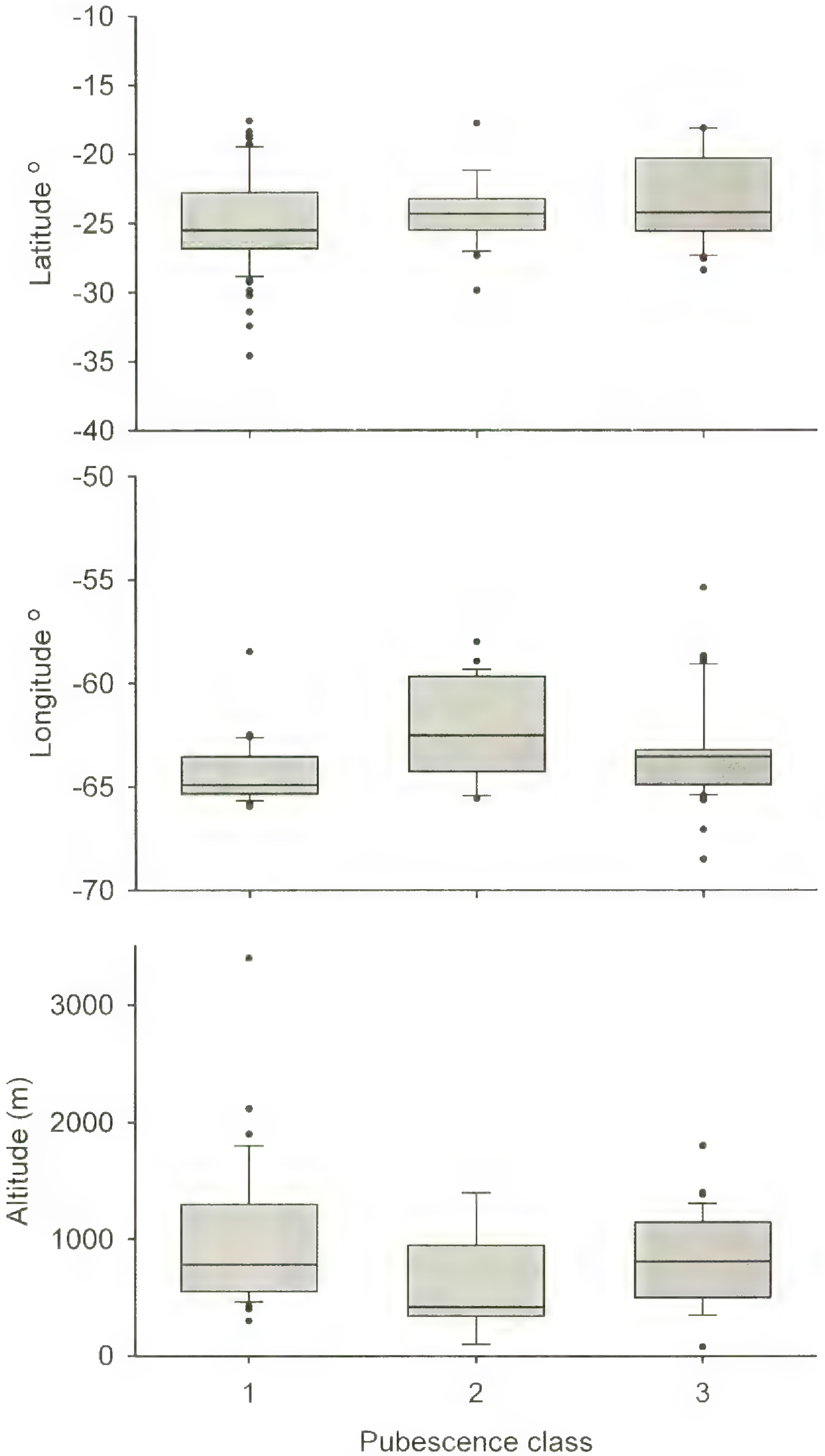

Figure 8. Environmental distribution of pubescence classes in *Solanum argentinum*. Box plots of the three classes of pubescence (1 = glabrous, 2 = tufts in vein axils, 3 = pubescence uniformly distributed on lamina) are plotted against latitude, longitude, and altitude above sea level. The central box in each class represents the central 50% of the data (with the median shown as a line). The outer bars represent 90% of the data, with the remaining 10% shown as outlying points. The figure shows that each of the three pubescence classes overlaps strongly in all three geographic dimensions. In each case, Kruskal-Wallis tests suggest that the medians do not differ ($P > 0.05$).

Río Itiyuro, *Krapovickas & Schinini 39269* (G); Dept. Capital, 20 km E de Salta, ruta Salta a Gral. Güemes, *Krapovickas & Cristóbal 30423* (G); Dept. Anta, 17 km NE de J. V. Gonzalez, camino a Puerta Blanca, *Krapovickas & Cristóbal 46303* (NY); Dept. Anta, 1ra. sección, El Dorado, *Luna 1049* (MO); Dept. Metán, Metán, *Malvárez 155* (G); Dept. Anta, Ceibalito, *Malvárez 305* (W); Dept. Orán, Senda Hachada, *Malvárez 497* (W); Dept. Capital, Capital, *Meyer 3663* (F); Dept. Cerrillos, Cerrillos, *Meyer 3652* (F); Dept. Anta, León Pozo, 30 km al W de Piquete Cabodo, *Meyer 9900* (BM, F, NY); Dept. Orán, Orán, *Meyer 5048* (F); Dept. Anta, Coronel Olleros, *Novara 527* (G [2], MO); Dept. Capital, Salta, sobre el FFCC a Quijano a la altura de calle Leguizamón, *Novara 535* (G); ruta 68 km 83 entre Alemanía y el Guayacán, *Novara 4572* (G); Dept. Guachipas, Alemanía, alrededores del pueblo, *Novara 4856* (G [2]); Dept. La Viña, Talapampa, en los alrededores de la hostería, ruta 68 Km 93, *Novara 5625* (G [2]); Dept. Capital, Chachapoyas, Sierra de Vélez, cerros de la Universidad Católica, *Novara 5892* (G [2]); Dept. La Viña, adyacencias del digue Puerta de Diaz, 4–6 km al W de Coronel Moldes, *Novara 7248* (G); Dept. Cerrillos, Finca Santa Margarita, Ruta 21, 4 km al N de San Agustin, *Novara 7755* (G [2]); Dept. Orán, Orán, *O'Donell 3135* (BM); Dept. Campo Santo, antes de Cabeza de Buey, *O'Donell 4357* (MO); Dept. Orán, Orán, *Pierotti 2* (F); Dept. Rosario de Lerma, Quebrada del Toro, *Ruthsatz 364/2* (MO); Dept. Anta, El Piquete, *Ragonese 274* (US); Talavera, *Schulz 2041* (NY); Dept. Chicoana, Dique Cabra Corral, en las Serranas del Dique, *Varela 1545* (NY); Dept. Guachipas, Alemania, *Venturi 9818* (BM, MO, US). **Santa Fé:** Dept. 9 de Julio, entre El Boliche del Turco y el Gato Colorado, *Ragonese 3000* (US). **Santiago del Estero:** betw. Brea Pozo and Río Huayco Hondo, Ferrocarril Central de Argentina, *Bartlett 19750* (US); Dept. Robles, Vilmes, *Bartlett 20474* (US); Dept. Capital, Capital, *Bianchi 12* (F, W); Dept. Silípica, El Mojón, *Gramaja 16* (BM, US); Dept. Capital, Mal Paso, *Huidobro 3045* (W); Dept. Robles, Fernandez, *Krapovickas 27157* (G, MO); Dept. Pellegrini, El Tigre, *Luno 24* (W); Dept. Robles, Turena, *Maldonado 227* (F, US); Dept. Copo, Urutaú, *Malvárez 653* (MO); Dept. Copo, Pampa de Los Guanocos, *Malvárez 691* (W); Dept. Guasayán, El Cevilas, Mar. 1944, *Pierotti s.n.* (US); Dept. Pellegrini, Estancia El Remate, *Venturi 5797* (BM, F, MO, US). **Tucumán:** El Molle, *Castellanos 14825* (US); Dept. Leales, Los Puestos, *Krapovickas & Cristóbal 27143* (MO); Dept. Capital, s. loc., *Lillo 3343* (G [2], US); rd. Tucumán to Racas, railroad bridge in open country, *Mexia 4341* (MO), *Mexia 7834* (BM, MO, US); Burruyacú, Taruca Pampa, Finca San Augine, *Norrbom 91A3* (US); Dept. Cruz Alta, Las Cejas, *O'Donell 4321* (NY); Dept. Leales, Las Enuncijadas, *Ousset 112* (F); Tapia, *Rodriguez 249* (W); s. loc., *Stuckert 8170* (G); Dept. Burruyacú, s. loc., *Stuckert 18156* (G), *Stuckert 19721* (G); Dept. Capital, Muñecas, *Venturi 2434* (US); Dept. Trancas, Vipos, *Venturi 2655* (US); Dept. Trancas, Leocario Paz, *Venturi 7774* (US); Dept. Burroyacu, Cerro del Campo, *Venturi 7857* (MO, US). BOLIVIA. **Chuquisaca:** prov. Azero, 5 km N de Carandaytí, *Krapovickas 31246* (MO); prov. Oropeza, Chataquila, *Murguia 379* (NY); prov. Luis Calvo, proximo al lugar de el Palmar, *Murguia R. 536* (NY); prov. Luis Calvo, El Salvador, zona central, *Penseiro 4323* (MO, NY). **Gran Chaco:** Cururenda, 200 m, *Cárdenas 2525* (US [2]). **Santa Cruz:** prov. Cordillera, Santa Cruz, ca. 200 km hacia el S, Proyecto Abapó Izozóg, cerca al Río Grande, *Beck 6459* (NY); prov. Cordillera, Aguarati Izozog, en las grandes dunas del Río Parapetí, 500 m al S de Aguarati, *Bourdy 2017* (NY); prov. Cordillera, Camiri, *Cárdenas et al. 4754* (US); prov. Chiquitos, 7 km al E de la planta de gas de Río Grande, sobre la brecha del gasoducto, *Fuentes & Novarro 1958* (MO); prov. Cordillera, Cuevo, adentro la sierra cerca la planchada antigua de Chevron, *Jardim & Roas-Hurtado 1550* (NY); prov. Cordillera, Boyuibe, del Pueldo, 11 km N del Pueblo, *Killeen et al. 4209* (NY); prov. Cordillera, 3 km NW from Camiri, *Kuroiwa & Maeda 2332* (NY); prov. Cordillera, Alto Parapetí, orilla del Río Parapetí, *Michel 2* (MO, NY); prov. Cordillera, E & NE sides of Camiri, along Río Parapetí, *Nee 35288* (NY); prov. Cordillera, 2–3 km NW of Boyuibe, along abandoned railroad to Cuevo, *Nee 35317* (MO, NY); prov. Cordillera, in Boyuibe, *Nee 35330* (NY); Prov. Andrés Ibáñez, city of Santa Cruz, *Nee 40420* (MO, NY); Prov. Andrés Ibáñez, Basilio, on rd. from Santa Cruz to Camiri, *Nee 44597* (BM, MO, NY, US); prov. Cordillera, around hwy. and railroad bridges over Río Seco on N side of settlement of Río Seco, along new hwy. from Santa Cruz to Abapó, *Nee 49083* (NY); prov. Cordillera, 6 km (by air) S of center of Camiri, along dirt rd. to Comunidad Yuti, *Nee 51132* (G, NY); prov. Cordillera, 2 km SW of center of Camiri, on SW side of Camiri, *Nee 51149* (NY); prov. Cordillera, 7 km S of Salinas on hwy. from Camiri to Cuevo, *Nee 51171* (NY); prov. Cordillera, along rd. from Camiri to Ipatí, 18.5 km N of bridge over Río Parapetí on N side of Camiri, *Nee 51320* (G [2], NY,); prov. Cordillera, along rd. from Camiri to Ipatí, 9.5 km N of bridge over Río Parapetí on N side of Camiri, *Nee 51337* (NY); Prov. Andrés Ibáñez, Basilio, *Nee et al. 51722* (BM, G [2], NY); prov. Cordillera, S side of bridge over Río Seco in town of Río Seco, *Nee et al. 51723* (BM, NY); prov. Florida, along hwy. from Mairana to Mataral, 3 km NE of bridge at Los Negros, *Nee et al. 52101* (BM); prov. Cordillera, La Brecha, Bañados de Izozog, alrededores del hospital y 3 km camino hacia Río Parpetí, *Vargas 1052* (MO, NY). **Tarija:** prov. Avilés, Sunari Pampa, cerca Colon N, *Bastian 1347* (NY); prov. Arce, 31 km S of jct. of rd. to Entre Ríos, on rd. to Padcaya, *Solomon 10579* (MO, US); ca. 25 km S of Tarija toward Padcaya, *Wood 9529* (NY). PARAGUAY. Jardín Botánico, *Rojas 14486a* (W). **Alto Paraná:** Río Atabó, *Itaipú Binacional 668* (MO). **Boquerón:** Puesto Uno, Pozo Hondo, a orillas de Río Pilcomayo, *Degen & Mereles 3149* (FCQ, MO [2]); a 22 km sobre Picada Mistolar de la Picada 4ta. División de Infantería, Pedro P. Peña, *Degen & Mereles 3176* (FCQ); zona del canal Paraguayo, en el Pilcomayo, *Degen & Mereles 3179* (MO); Pozo Hondo, orillas del Río Pilcomayo, *Mereles & Degen 5570* (FCQ, MO); Tyto. Misión-Estancia Oasis, 6 km S del desvío a Pozo Hondo, *Mereles & Degen 5616* (FCQ); entrada al Puesto de Buenos Aires, *Mereles & Degen 5642* (FCQ, MO); Estancia María Esther, borde de la Cañada Madrid, *Mereles & Degen 5859* (FCQ); cercanías del Río Pilcomayo, *Mereles & Degen 6114* (FCQ); Avalo Sanchez-Estancia Brusquetti, *Soria 364* (FCQ); Retiro Aloncito (Estancia Tinfunque), Campo Montaño, 12 km al N del Retiro Avalos Sanchez, *Spichinger RS2185* (FCQ); Estación Experimental Isla Poí, *Vanni et al. 2362* (NY). **Presidente Hayes:** de Pozo Colorado versus Fortin Gen. Díaz, *Bernardi 20283* (MO, NY); Estancia Bruschetti Avalos Sánchez, *Soria 364* (FCQ, MO).

6. **Solanum delicatulum** L. B. Sm. & Downs, Phytologia 10: 424. 1964. TYPE: Brazil. Santa Catarina: Itajaí, Cunhas, 10 m, 8 Feb. 1955, *R. Klein 1131* (holotype, HBR not seen; isotype, US 00027002 [fragm.]!]).

Solanum pavimenti L. B. Sm. & Downs, Phytologia 10: 428. 1964. TYPE: Brazil. Santa Catarina: Papanduva, Lejeadinho, 800 m, 3 Jan. 1962, *P. Reitz & R. Klein 11438* (holotype, US!; isotypes, HBR not seen, NY!).

Herbs to subshrubs to small shrubs 0.5–1.5 m; stems slender and erect, ± densely pubescent with a mixture of simple and branched (dendritic) uniseriate trichomes ca. 0.5 mm, the trichomes closely appressed and ascending; bark pale yellowish; new growth densely pubscent like the stems with a mixture of simple and branched trichomes, especially on the veins; sympodial units difoliate, usually geminate, but occasionally the leaf pair distant, some nodes occasionally trifoliate. Leaves adaxially glabrous to sparsely pubescent on the veins and lamina with a mixture of simple and dendritic trichomes ca. 0.5 mm, abaxially sparsely to densely pubescent on the veins, more sparsely pubescent on the lamina, the trichomes simple or furcate-dendritic, ca. 0.5 mm; major leaves 3–7 × 1.4–3 cm, elliptic to ovate, the base acute, the margins entire, the apex acute; primary veins 6 to 7 pairs, drying yellowish; petioles 0.4–1 cm, glabrous to sparsely pubescent; minor leaves 1.5–2 × 1–1.6 cm, similar in shape to the major leaves but usually somewhat more orbicular, the base acute, the margins entire, the apex acute; petioles 0.2–0.4 cm. Inflorescences internodal or somewhat leaf-opposed, 0.2–0.5 cm, simple, with 2 to 3 flowers, sparsely pubescent with a mixture of trichomes like those of the stems, the peduncle 0.1–0.2 cm; pedicels 0.5–0.6 cm, less than 0.5 mm diam. at the base, ca. 1 mm diam. at the apex, nodding at anthesis; pubescent with simple and dendritic trichomes like those of the stems and inflorescences; pedicel scars closely spaced, overlapping at the tip of the inflorescence, plane with the rachis; buds globose, the corolla included in the calyx lobes. Flowers all perfect; calyx tube 1–1.5 mm, open-conical, the lobes 2–2.5 mm, long-triangular, sparsely pubescent with simple uniseriate trichomes; corolla 0.9–1 cm diam., white, lobed ca. 3/4 of the way to the base, the lobes 0.7–0.9 cm, deltate, planar to slightly reflexed at anthesis, glabrous or sparsely pubescent with scattered trichomes along the petal midveins and tips adaxially; filaments with the free portion ca. 0.5 mm, the tube less than 0.5 mm, glabrous; anthers 2–2.5 × ca. 1 mm, poridical at the tips, the pores lengthening to slits with age; ovary conical, glabrous, the style 4–5 mm, straight, glabrous, the stigma 2-lobed to bifid, the surfaces minutely papillate. Fruit a globose berry, 1–1.2 cm diam., red-orange at maturity, the pericarp thin and brittle; fruiting pedicels 0.5–0.7 cm, ca. 1 mm diam. at the base, woody and erect; seeds 3–4 × 2–3 mm, flattened-reniform, yellowish tan, the margins thickened, the surfaces minutely pitted. Chromosome number not known.

Distribution. Brazil (Santa Catarina and São Paulo), Argentina (Misiones and Corrientes), and eastern Paraguay (fide Gutiérrez et al., 2006), forests and forest edges at ca. 800 m.

Common name. "Canema mirim" (Smith & Downs, 1966).

Discussion. *Solanum delicatulum* was excluded from section *Geminata* (it was thought to be a member of section *Solanum* or a related group), and its synonym *S. pavimenti* included in the synonymy of *S. pseudocapsicum* by Knapp (2002a). Subsequent morphological studies of this rare species by L. A. Mentz (Mentz & Oliveira, 2004) have shown that it is a member of the section, and a member of the *S. pseudocapsicum* group with bright red fruit, and pedicels nodding in flower and erect in fruit. Some authors (G. E. Barboza, pers. comm.; Gutiérrez et al., 2006) regard *S. pavimenti* and *S. delicatulum* as distinct based on a suite of fruit and leaf characters that I think are part of the overall variation in *S. delicatulum*.

Studies by Gutiérrez et al. (2006) show that *Solanum delicatulum* is relatively widespread in Argentina and Brazil and occurs sporadically in eastern Paraguay, although I have seen no Paraguayan material. It is a plant of understory in forests and along streams, and can be assessed as Near Threatened (NT) or Vulnerable (VU), depending upon the circumscription of the species (as including *S. pavimenti*, as defined here or excluding it, as delimited by Gutiérrez et al., 2006).

Additional specimens examined. ARGENTINA. **Corrientes:** Dept. Berón de Austral, Berón de Austral, segunda S. Rural, *Ibarrola 3854* (W). **Misiones:** Arroyo Uruguaí, Km 10, *Partridge 61701* (US); Dpto. Gral. Manuel Belgrano, Parque Prov. Uruguaí, Ruta Prov. 19, 50 km de Wanda camino a Deseado, margen del Río Uruzú, *Morrone et al. 1951* (MO). BRAZIL. **São Paulo:** São Paulo, Parque do Estado, grounds of the Instituto de Botânica, 10 km S and 2.2 km E of center of São Paulo, Praça da Sé, *Eiten & Clayton 5790* (NY); Cidade Jardim, *Hoehne* (*SPF 10696*) *s.n.* (NY); Cidade Jardim, *Hoehne* (*SP 44929*) *s.n.* (NY).

Additions to the *Solanum nudum* Species Group

Shrubs, treelets, or small trees; young stems and leaves glabrous or pubescent, the trichomes simple or occasionally furcate or dendritic, often only on one side of the stem; sympodial units difoliate, usually geminate, usually anisophyllous. Leaves simple, elliptic to lanceolate, glabrous or pubescent, often with tufts of trichomes in the axils of the main veins abaxially, the trichomes simple or occasionally dendritic. Inflorescences opposite the leaves, usually simple, occasionally branched, glabrous or pubescent. Flowers white or greenish white, sometimes fleshy, the calyx lobes large and leafy to deltate, the corolla lobes

planar or slightly reflexed at anthesis; anthers usually quite stout. Fruit a green, hard berry, becoming yellowish at maturity; fruiting pedicel woody, deflexed; seeds flattened-reniform, pale tan to yellow.

Distribution. Members of the species group are often widespread in secondary habitats through the Neotropics, but some species are more local and occupy primary forest habitats.

7. Solanum chalmersii S. Knapp, Brittonia 58: 330. 2006. TYPE: Bolivia. La Paz. Prov. Sud Yungas: 7.5 km (by rd.) from Huancané on rd. to San Isidro, moist montane forest, 16°21′S, 67 30′W, 2225 m, 10 May 2001, *M. Nee, L. Bohs, S. Knapp & J. M. Mendoza F. 51777* (holotype, LPB!; isotypes, BM!, NY!, USZ!).

Shrubs or small trees 2–6 m tall; young stems and leaves densely white pubescent with simple, uniseriate trichomes ca. 1 mm, composed of 2 to 5 cells; older stems remaining densely white pubescent, occasionally glabrate; bark of the older stems and trunks pale yellowish white; sympodial units unifoliate or difoliate and geminate. Leaves elliptic to narrowly elliptic, chartaceous, evenly pubescent adaxially with simple uniseriate trichomes ca. 1 mm, densely pubescent abaxially with white uniseriate trichomes 1–1.5 mm, the trichomes denser on the veins; major leaves 9–17 × 3–6 cm, the base acute, the margins entire, the apex acute, rounded at the very tip; minor leaves 1.5–3 × 1–2 cm, differing from the major ones only in size, but occasionally somewhat rounder in outline; primary veins 9 to 12 pairs, raised adaxially, prominent and yellowish green abaxially; petioles 0.3–0.7 cm in major leaves, ca. 0.5 cm in minor leaves, densely pubescent. Inflorescences 1.5–5 cm, opposite the leaves or occasionally somewhat internodal, unbranched, with 10 to 20 flowers, densely white pubescent with simple uniseriate trichomes 0.5–1.5 mm, the peduncle 1–3 cm; pedicels 1–1.2 cm, tapering from the abrupt base of the calyx tube to a slender base 0.5–0.8 mm diam., articulated at the base; pedicel scars closely packed, often overlapping; buds when very young appearing globose, the corolla soon exserted from the calyx lobes, the buds later becoming obovoid just before anthesis. Flowers all perfect; calyx tube 1–1.5 mm, the lobes ca. 1 × 1 mm, deltate to broadly triangular, abruptly constricted to an elongate tip ca. 0.5 mm long, densely pubescent with simple, uniseriate trichomes like those of the rest of the inflorescence; corolla 1.5–2 cm diam., stellate, lobed 2/3 to 3/4 of the way to the base, white or sometimes tinged purplish, the lobes ca. 1 × 0.5 mm, ovate-lanceolate, reflexed at anthesis, densely and evenly pubescent abaxially with simple uniseriate trichomes ca. 0.5 mm, glabrous adaxially except for the densely papillose margins, the tips of the lobes densely papillose and somewhat cucullate; filaments with the free portion 0.8–1 mm, the tube 1–2 mm, glabrous, with small teeth arising between the free portions of the filaments; anthers 4–5 × 1–1.5 mm, oblong, slightly sagittate at base, the pores teardrop-shaped, opening into longitudinal slits with age; glabrous; ovary glabrous; style 6–7 × ca. 0.05 mm, cylindrical, straight, glabrous, the stigma capitate. Fruit a globose berry 1–1.2 cm diam., green, glabrous fruiting pedicels 1.5–2.2 cm, 0.5–1 mm diam. at the base, pendent, woody, the fruiting calyx not accrescent, the lobes ca. 1 × 1 mm, brittle and somewhat patent; seeds 3–3.5 × 2–2.5 mm, flattened-reniform, pale yellow in dry material, the surfaces minutely pitted, the margins incrassate and darker yellow.

Distribution. In the understory of montane forest in northern Bolivia, on eastern Andean slopes from 1900–2200 m. Plants of *Solanum chalmersii* grow both in the forest understory and in disturbed areas along roads and streams, attaining higher population densities in open areas.

Discussion. Solanum chalmersii was mentioned as a probable new species in Knapp (2002a), but at that time, material was not sufficient to distinguish it from other similar species in the large *S. nudum* species group, and good fruiting material was not available in order to place it confidently. *Solanum chalmersii* possesses the flattened-reniform, pale-colored seeds, simple uniseriate trichomes, and more or less closely spaced pedicel scars typical of the *S. nudum* species group. It is most similar to *S. acuminatum* Ruiz & Pav. of central Peru to Bolivia, with which it is nearly sympatric, but differs in its uniform covering of simple uniseriate trichomes (usually confined to the vein axils in *S. acuminatum*), its flowers with longer anthers with sagittate bases, and in its longer fruiting pedicels. In Bolivia, where the two species are sympatric, *S. acuminatum* occurs at higher elevations than *S. chalmersii*.

Solanum chalmersii, despite its small overall range, is common where it occurs and forms quite dense thickets at the margins of roads and streams. It can be assessed (IUCN, 2001) as Vulnerable (VU) due to this restricted range (< 5000 km^2) and occurrence in only a few locations, but it is of less concern due to its probably weedy nature.

Additional specimens examined. BOLIVIA. **La Paz:** Prov. Sud Yungas: de Chulumani hacia el N unos 5 km hacia Irupana, entrando hacia Apa Apa, *Beck 24480* (NY); Sirupaya vic. de Yanacachi, *Buchtien 315* (NY); along rd. through primary cloud forest, 7.0–9.4 km NE of (above) Huancané, *Luteyn & Dorr 13722* (BM, LPB, NY); 5.2 km (by

rd.) from Huancané on rd. to San Isidro, moist montane forest, *Nee et al. 51773* (BM, LPB, NY, USZ); rd. betw. Unduavi & Puente Villa, around hotel Castillo del Chaco & down to the Río Unduavi, *Nee et al. 51795* (BM, LPB, NY, USZ); Yanacachi, 3.5 km hacia Chojlla (as Chajlla), *Seidel & Richter 851* (LPB, NY); Cantón Yanacachi, Mina Chollja, camino a Kacapi, *Siñani 261* (LPB); ca. 6 km along rd. from Huancané to San Isidro, *Wood 9955* (K).

8. Solanum monanthemon S. Knapp, Brittonia 58: 332. 2006. TYPE: Bolivia. Santa Cruz. Prov. Caballero: Parque Nacional Amboró, Cerro Bravo, próxima a las juntas del Río Alizar y Amparo (20 km al NW de San Juan de Potrero), 17°57′S, 64°54′W, 2000 m, 10–14 Apr. 1994, *I. Vargas C., D. Ayala & J. Quiroga 3138* (holotype, LPB!; isotypes, BM!, NY!, USZ!).

Shrubs to 1.5 m tall; young stems glabrous and somewhat shiny; older stems glabrous; bark of older stems shiny reddish brown; sympodial units difoliate and geminate, the minor leaf often deciduous and nodes thus appearing unifoliate. Leaves ovate to elliptic or narrowly elliptic, chartaceous, glabrous and shiny adaxially, glabrous abaxially but with tufts of white simple uniseriate trichomes in the axils of the veins, the trichomes ca. 0.5 mm, very thin and interlaced, arising from the lamina; major leaves 7–11 × 2–4 cm, the base acute to slightly attenuate onto the petiole, the margins entire, the apex acute to long-acuminate and rounded at the very tip; minor leaves 2–5 × 1–2.7 cm, differing from the major leaves only in size; primary veins 5 to 6 pairs, only the midrib raised adaxially, the veins reddish brown abaxially; petioles 0.5–1 cm in major leaves, 0.5–0.7 cm in minor leaves, glabrous. Inflorescences consisting of a single flower (occasionally the scar of a second flower apparently present), opposite the leaves or occasionally somewhat internodal, glabrous, the peduncle and rachis absent or the pedicel appearing jointed 0.6–0.9 cm from the base; pedicels 3–3.5 cm (to 4 cm if measured to the base of the inflorescence), deflexed, tapering from the base of the calyx tube to a slender base ca. 0.2 mm diam., articulated at the base; buds when very young appearing globose, the corolla included within the elongate calyx lobes, the buds remaining globose until anthesis. Flowers apparently all perfect; calyx tube 1–1.5 mm, the lobes 3–5 × ca. 0.5 mm, long-triangular, glabrous; corolla 0.8–1 cm diam., stellate, lobed ca. 2/3 of the way to the base, white, the lobes ca. 0.5 × 0.3 mm at base, ovate-lanceolate, probably reflexed at anthesis, glabrous abaxially and adaxially except for the minutely papillose tips; filaments with the free portion ca. 0.5 mm, the tube ca. 0.5 mm, glabrous; anthers 2.5–3 × ca. 1 mm, oblong, slightly sagittate at base, connivent, yellow, the pores teardrop-shaped, opening into longitudinal slits with age; ovary glabrous; style 3–4 × ca. 0.1 mm, cylindrical, straight, glabrous, the stigma capitate. Fruit a globose berry, 1–1.2 cm diam. (immature), green, glabrous; fruiting pedicel 4.5–5 cm, 0.5–1 mm diam. at the base, ca. 3 mm diam. at the apex, pendent, slender, the fruiting calyx slightly accrescent, the lobes 5–6 × ca. 0.5 mm; seeds not known from mature fruit.

Distribution. Only known from two specimens collected in cloud forests in Parque Nacional Amboro and Parque Nacional Carrasco on the eastern slopes of the Andes in Bolivia at ca. 2000 m. The cloud forests where *Solanum monanthemon* grows have Podocarpaceae and Myrtaceae as canopy trees (fide label on *Vargas et al. 3138*).

Discussion. Specimens of *Solanum monanthemon* have occasionally been identified as the more common Bolivian species *S. trichoneuron* Lillo. It is superficially similar to that species, but differs in its dark bark and single-flowered inflorescences, which look like a single flower on an articulate pedicel arising opposite the geminate leaves. The apparent articulation is actually the joint between the inflorescence axis and the pedicel, as evidenced by some inflorescences with minute second buds that apparently abort early in inflorescence development. Many more collections of *S. monanthemon* are needed to investigate this phenomenon fully. Mature fruits of *S. monanthemon* are not known, so species group placement is tentative at present. Overall morphology (tufts of hairs in the vein axils, short anthers) suggests *S. monanthemon* belongs to the *S. nudum* species group, as accepted here, but this can be misleading (e.g., *S. smithii* S. Knapp of the *S. arenarium* Sendtn. species group also possesses these hair tufts; see Knapp, 2002a).

Solanum monanthemon can be distinguished from other species with tufts of hairs in the abaxial vein axils by its single-flowered inflorescences and its tree-like habit. It also apparently grows as isolated individuals in dense cloud forests, unlike many of the other members of the *S. nudum* species group that are plants of open areas along streams and roads both in Bolivia and throughout the Neotropics.

Solanum monanthemon is known only from two collections and, thus, cannot be assessed with confidence using the IUCN criteria (IUCN, 2001), but is likely to be of conservation concern. A conservative preliminary conservation assessment would be Endangered (EN) based on the few collections (small area of occupancy) some hundreds of kilometers apart (indicating a larger extent of occurrence of < 5000 km^2), but further searches for this apparently rare species are a priority. Its

occurrence in the Parques Nacionales Amboro and Carrasco may afford it some protection.

Additional specimens examined. BOLIVIA. **Cochabamba:** Prov. Carrasco, Sehuencas, Río Fuerte cerca puente [El Puente?], Parque Nacional Carrasco, *Ibisch & Ibisch 93.1477* (LPB).

9. Solanum pabstii L. B. Sm. & Downs, Phytologia 10: 427. 1964. TYPE: Brazil. Santa Catarina: Lajes–São Joaquim, bank of the Rio Lavatudo, 1050 m, 22 Oct. 1961, *G. Pabst 6200 & E. Pereira 6373* (holotype, US 00027014!; isotype, HBR not seen). Figure 9.

Treelets or small trees 3–5 m; stems erect, glabrous; bark dark on young stems, yellowish on older stems; new growth glabrous or occasionally minutely papillate; sympodial units plurifoliate. Leaves 3.5–10.5 × 1–2.5 cm, narrowly elliptic to lanceolate, glabrous adaxially, the vein axils with resinous dots, glabrous and with distinct pit-like domatia in the vein axils abaxially, the base acute to attenuate, the margins entire, slightly revolute, the apex acute to acuminate; primary veins 6 to 8 pairs, impressed adaxially; petioles 0.2–1 cm, glabrous. Inflorescences internodal to apparently terminal, 2–8 cm, 3 to 4 times branched, with 3 to 50 flowers, glabrous, thin and brittle in dry specimens, the peduncle 1–2 cm; pedicels 1.3–1.5 cm, ca. 0.1 mm diam. at the base, ca. 0.2 mm diam. at the apex, glabrous, ± nodding at anthesis, articulating at the base; pedicel scars irregularly spaced 1–10 mm apart, plane with the rachis; buds narrowly ellipsoid, the corolla strongly exserted from the calyx. Flowers all perfect; calyx tube 1.5–2 mm, conical, the lobes 1–1.2 mm, deltate, apiculate, the margins membranous and paler, glabrous; corolla 1.3–1.9 cm diam., white, lobed ca. 2/3 of the way to the base, the lobes 0.8–1.3 cm, broadly deltate, campanulate at anthesis, the tips densely papillate; filaments with the free portion 1–2 mm, the tube absent, glabrous; anthers 2.5–4 × 1–1.5 mm, poricidal at the tips, the pores lengthening to slits with age; ovary glabrous, conical, the style 5–6 mm, straight, glabrous, the stigma clavate to slightly 2-lobed, the surface minutely papillate. Fruit a globose berry, 0.8–0.9 cm diam., green when ripe; fruiting pedicels ca. 2 cm, not markedly enlarged at the apex; seeds not known. Chromosome number not known.

Distribution. In the Brazilian states of Santa Catarina, Paraná, and Rio Grande do Sul, in Atlantic Forest and forest margins, from 600–1300 m.

Common name. "Canema" (Smith & Downs, 1966).

Discussion. *Solanum pabstii* is a very striking species, with its large, open, many-branched inflorescences of relatively large, white flowers. The inflorescence axes of *S. pabstii* are extremely thin and filiform, and are brittle on herbarium specimens. Specimens of *S. pabstii* have been misidentified as *S. pseudoquina* A. St.-Hil., another species of the *S. nudum* species group occurring in southern Brazil. The two species share filiform inflorescence axes (although the inflorescences of *S. pabstii* are much more branched) and leaves that dry pale green on the herbarium sheet. They differ in flower morphology, with *S. pabstii* having all anthers of equal size and dehiscence of pores lengthening to slits with age, and *S. pseudoquina* having markedly unequal anthers that dehisce by tiny pores that never lengthen to slits. *Solanum pseudoquina* has tufts of uniseriate trichomes in the vein axils on the abaxial leaf surfaces, while *S. pabstii* has small resinous patches on the new growth in the vein axils that develop into cavities with resinous margins in mature leaves (see Fig. 10). The cavities are probably domatia (sensu O'Dowd & Willson, 1991) and are a distinctive feature of the species, found nowhere else in the Geminata clade, and to my knowledge nowhere else in *Solanum*. Not all mature leaves develop the cavities to their full extent, but at least some leaves on every specimen examined had them. The ecological function and development of these cavities will be an interesting study in plant-insect interaction, should they be found to house mites.

Although the seeds of *S. pabstii* are not known at present, I suspect that they might be flattened-reniform with enlarged margins. *Solanum pabstii* conforms to other characters of the *S. nudum* species group, but as yet has not been included in phylogenetic analyses.

Solanum pabstii is a species of disturbed habitats and of relatively wide distribution and, thus, can be assigned a preliminary conservation status of Vulnerable (VU) due to its relatively narrow global distribution (< 20,000 km^2) using the IUCN criteria (IUCN, 2001). Further work on its specific distribution within the threatened interior Atlantic Forest of Brazil may indicate that it may be of further concern.

Additional specimens examined. BRAZIL. **Paraná:** Palmas, *Hatschbach 15030* (F, NY, US); Clevelandia, *Hatschbach 22707* (F, MO, NY); Mun. Lapa, Segundo Faxinal, trevo para Monte Alegre, *Ribas & Silva 190* (BM). **Rio Grande do Sul:** Aparades da Serra, *Pabst 6281 & Pereira 6454* (US); São Salvador, *Sehnem 2203* (NY, US); São Salvador, Montenegro, *Sehnem 3934* (F, NY, US); Estrada B. Ouro-Riozinho, *Sobral et al. ICN 111138* (NY); Mun. São Francisco do Paula–RS 235, *Wasum 270* (G). **Santa Catarina:** Mun. Lajes, Painel, Lajes, *Klein 4567* (NY, US); Mun. Urubici, cachoeira do Avencal, 29 Mar. 2001, *Hatschbach et al. 72547* (NY); Mun. Urubici, Vacas Gordas, *Mentz & Sobral 188* (NY); BR-2, planalto Santarinense, perto Sta. Cecilia, *Pabst 6123 & Pereira* (K, US); Mun. Agua Doce,

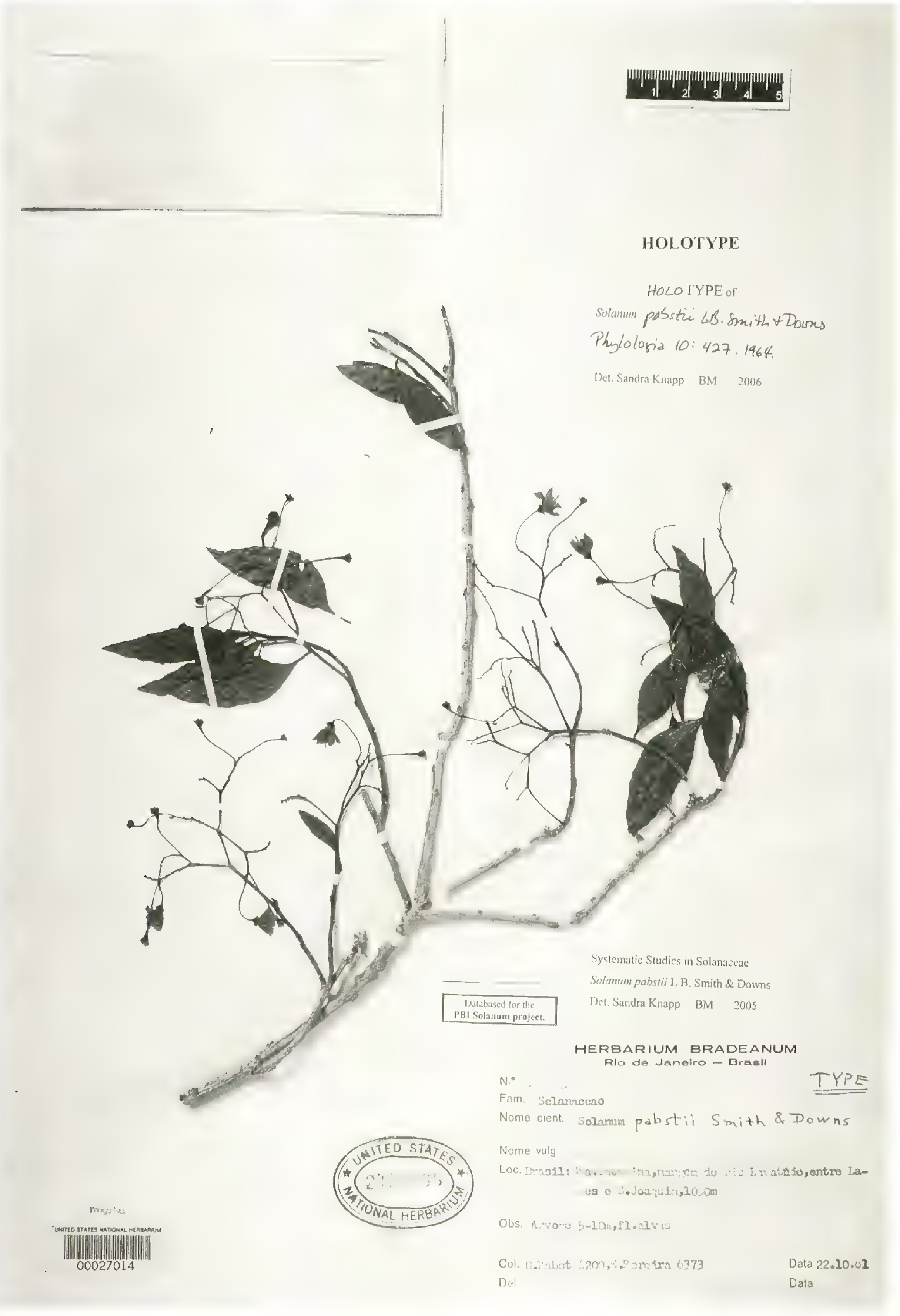

Figure 9. Holotype of *Solanum pabstii* (*Pabst 6200 & Pereira 6373* [US]).

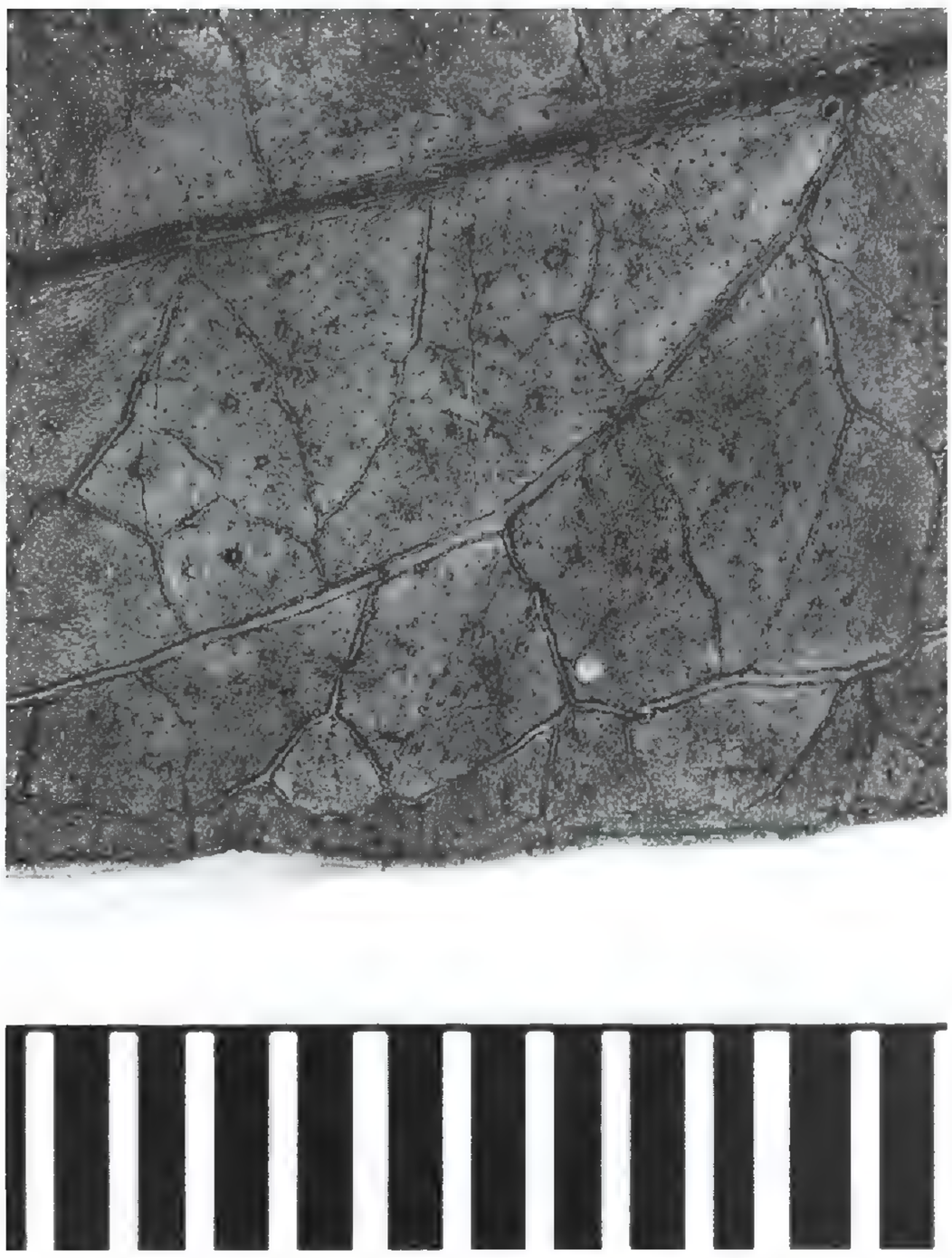

Figure 10. Detail of domatia from leaf undersurface of *Solanum pabstii* (from *Hatschbach 22707*, MO). Scale bar in millimeters.

Campos de Palmas, 24 km SE of Horizonte (Paraná), *Smith & Klein 13489* (MO, NY, US); Bom Jardim da Serra, estrada B. Jardim–S. Joaquim, Km 29, *Stehmann & Dutilh 1549* (NY).

Addition to the *Solanum leucocarpon* Species Group

Shrubs or small trees; young stems and leaves glabrous or pubescent with simple, uniseriate trichomes; sympodial units difoliate and geminate or plurifoliate, often anisophyllous. Leaves simple, elliptic to occasionally ovate, glabrous or variously pubescent with simple, uniseriate trichomes, these occasionally dendritic in some populations. Inflorescences opposite the leaves or somewhat internodal, simple or branched, glabrous or pubescent like the stems and leaves. Flowers white, fleshy, and relatively large, the corolla lobes planar at anthesis. Fruit green or yellowish green at maturity; fruiting pedicels erect or slightly deflexed, usually enlarged just below the berry, woody; seeds flattened-reniform, the margins incrassate, paler, reddish brown to yellowish tan.

Distribution. Species in the group are distributed in secondary habitats in South America, from Panama to Brazil.

10. Solanum evonymoides Sendtn. in Mart., Flora 24, Beibl. 2(5): 87. 1841. TYPE: Brazil. Bahia: "Ilheos," Nov. 1839, *C. Martius 625* (lectotype, designated here, NY!; isotypes BM!, G!, G-DC!, K!, MO!, P! [Morton neg. 8189]). Figure 11.

Shrubs or small trees 1–5 m; stems erect, stout and hollow (shattering in dry specimens), glabrous and shiny, usually dark reddish; bark dark reddish brown; new growth glabrous or sparsely red-papillate; sympodial units difoliate, not geminate. Leaves (7–) 10–25 × (1.5–)3.2–6 cm, elliptic, glabrous and shiny adaxially, glabrous or with sparse simple uniseriate trichomes 0.5–1 mm in the vein axils and along the midrib abaxially at the junctions of veins and lamina, the base attenuate, the margins entire, the apex acuminate; primary veins 9 to 13 pairs, drying yellowish; petioles 1.5–6 cm, glabrous. Inflorescences leaf-opposed or apparently (but structurally not in axils) in branch forks, (1.5–)3–4 cm, 3 to 4 times branched, with 10 to 40 flowers, glabrous, the peduncle absent and the inflorescence branching at the very base, or 0.1 to rarely 1 cm; pedicels 1.5–2.2 cm, ca. 1 mm diam. at the base, ca. 2 mm diam. at the apex, slender, nodding to ± erect at anthesis, completely glabrous, shiny, articulating at the base; pedicel scars irregularly spaced 2–6 mm apart, plane with the rachis; buds fusiform with the tip strongly pointed, strongly exserted from the calyx. Flowers apparently all perfect; calyx tube 1–1.5 mm, broadly conical, the lobes 1.5–2 mm, deltate, glabrous but with a dense tuft of uniseriate trichomes on the swollen lobe tip; corolla 2.1–2.6 cm diam., white, sweet-scented (fide *Kallunki et al. 706A*), lobed ca. 3/4 of the way to the base, the lobes 1.6–2.2 cm, planar at anthesis, densely papillate on the margins on both surfaces, hooked and cucullate at the apex; filaments with the free portion 0.5–1 mm, the tube ca. 0.25 mm, glabrous; anthers 3.5–5 × 1.5–2 mm, poricidal at the tips, the pores lengthening to slits with age; ovary glabrous, conical, the style 6–9 mm, straight, glabrous, the stigma clavate or occasionally (São Paulo populations) 2-lobed, the surfaces minutely papillate. Fruit a globose berry, 2–3 cm diam., green at maturity, the pulp sticky; fruiting pedicels 2.5–3 cm, ca. 1.5 mm diam. at the base, 3–4 mm diam. at the apex, woody and pendent; seeds 4–5 × 3.5–4 mm, flattened-reniform to ± ovoid, the margins not markedly thickened, pale yellow or white, the surfaces minutely pitted, sometimes appearing hairy. Chromosome number not known.

Distribution. Coastal Atlantic Forest of southeastern Brazil, in the states of Bahia, Espírito Santo, Rio de Janeiro, and São Paulo, from 0–550(–1800) m.

Common names. Brazil. Bahia: "caiçara" (*Lima & Messias Santos 144*), "pimientera brava" (*Santos 73*).

Discussion. *Solanum evonymoides* is a distinctive species, with its shiny, glabrous leaves and its open, highly branched inflorescences with large, white flowers. Label data indicate that the flowers are sweetly scented (*Kalllunki et al. 706A*). The cucullate, strongly hooked petal lobe apices are distinctive in *S. evonymoides*. Overall morphology suggests it is best placed in the *S. leucocarpon* Dunal species group, with other species possessing large flowers, rather large open inflorescences, and flattened seeds. Within that group it is most similar to *S. alatirameum* Bitter, also of southeastern Brazil, but can be distinguished from that species by its petiolate (rather than sessile) leaves and larger berries.

Confusion over the identity of *Solanum evonymoides* has existed for some time, with specimens being annotated as a variety of taxa. Specimens of *S. evonymoides* have been annotated as *S. glomuliflorum* Sendtn., a species of uncertain identity (most probably a species of *Aureliana* Sendtn. or *Athenaea* Sendtn., see Knapp, 2002a). The later name *S. evonymoides* J. Rémy in Gay (1846), a species of coastal southern Chile, is a synonym of *S. valdiviense* Dunal (Knapp, in prep.), a member of *Solanum* sect. *Holophylla* s. str. (see Knapp, 1989) and the Dulcamaroid clade sensu Bohs (2005). *Solanum cordioides* S. Knapp has been annotated as *S. evonymoides* in several herbaria (see Knapp, 2002a), but the two species differ in flower morphology (a smaller corolla 0.6–0.8 cm diam. and green in *S. cordioides* vs. 2.1–2.6 cm diam. and white with cucullate, hooked apices in *S. evonymoides*) and fruit size (1–1.2 cm diam. in *S. cordioides* vs. 2–3 cm diam. in *S. evonymoides*). *Solanum evonymoides* has also been confused with *S. sycocarpum* Mart. & Sendtn. (ex *Cyphomandra* Sendtn.), with which it is very similar morphologically. *Solanum evonymoides* differs in its uniformly reddish bark, the pedicels abscising flush with the inflorescence rachis and thus not leaving conspicuous pegs, and in its anthers without an enlarged connective (typical of members of the Cyphomandra clade, sensu Bohs, 1994, 2005; Weese & Bohs, 2007).

Populations of *S. evonymoides* that have smaller, shinier leaves and are altogether smaller in all parts (flowers included) from São Paulo and Rio de Janeiro states in Brazil are here treated as a geographical variant, but may warrant distinction at the species level (*Custodio Filho 265*; *Hoehne 3042*; *Martinelli et al. 13274, 13360*; *Martins et al. 12361*; *Pirani et al. 3028A*). Some of these plants with very coriaceous leaves and smaller flowers are from higher elevations (1000–1800 m) and are components of saxicolous vegetation (*Martinelli et al. 13274*). These plants, despite their marked habitat differences from other populations of *S. evonymoides*, share the key characteristics of the species: glabrous foliage, difoliate but not geminate sympodia, branched inflorescences with white flowers, petals with cucullate, hooked apices, and green fruits at maturity.

Solanum evonymoides is relatively widespread in the Atlantic Forest of coastal southern Brazil and appears to be relatively common where it does occur. However, the restricted nature of its habitat and the threats to this habitat make it necessary to monitor the conservation status of this species. Based on current herbarium specimen data, it can be assigned a status (IUCN, 2001) of Vulnerable (VU) (extent of occurrence $< 20{,}000$ km^2 and occurring in approximately 10 locations, some of which are fragmented), with monitoring across the whole species range and field assessment of the southern populations both being priorities.

The holotype of *Solanum evonymoides* was destroyed in Berlin, so the NY sheet is chosen as lectotype (Fig. 11) as it is annotated with the exact collecting locality (Ilheus) and date (1839). It has several open flowers and is a good match for the destroyed B sheet (F neg. 2817); isolectotypes are widely distributed.

Additional specimens examined. BRAZIL. s. loc., *Anon. 625* (GH), *Sellow s.n.* (BM), *Sellow 819* (BM). **Bahia:** Eunapolis–Itamaraju, *Almeida 16* (US); Mun. Uruçuca, dist. Serra Grande, 7.3 km na estrada Serra Grande–Itacaré, conjunto Faz. Santa Cruz, *Amorim 653* (K, NY); s. loc., *Blanchet 3096A* (G, W), *Blanchet 3096* (G [2]); Mun. Santa Cruz Cabrália, Est. Ecológica do Pau–Brasil, *Brito & Da Vinha 117* (F); Mun. Una, Riberão da Caveria, Serra Javi, ramal com entrada no Km 11 da rodovia São Jose–Una, lado S, 6 km do S de entrada, *Brito & Judzeiwicz 3986* (NY, US); Mun. Itamaraju, rod. Itamaraju–Teixeira de Freitas (BR 101), 3 km de Itamaraju, Faz. Chapadão, *Callejas et al. 1618* (NY); Mun. Ilhéus, Km 18 Rod. Ilhéus–Itacaré, Bom Retiro, *Carvalho et al. 6672* (BM); Mun. Itacaré, loteamento de Marambaia, ca. 6 km SW de Itacaré na estrada para BR-101, *Carvalho et al. 4121* (K, NY); Mun. Una, Fazenda Piedade, rod. São Jose–Una, a 9 km do BR-101, *dos Santos & Alves 221* (NY); Ubaitaba, Ibirapitanga, *Dos Santos 1051* (US); Mun. Santa Cruz Cabrália, Est. Ecol. Pau–Brasil, CEPLAC, *Euponino & Da Vinha 481* (NY); Mun. Santa Cruz Cabrália, Faz. Guiberti, *Lima 144* (NY); Mun. Belmonte, Estacão Exp. Gregorio Bondar, Km 58 da rodavia Belmonte–Itapebi, *Mattos Silva et al. 376* (F); Mun. Una, Km 27 da Rod. São Jose–Una, proximo a faz. Piedade, *Mattos Silva et al. 1298* (F); Mun. Una, povoado de Comandatuba, 17 km ao S de Una, *Mattos Silva et al. 1378* (F, MO); Mun. Itamaraju, ca. 5 km a W de Itamaraju, *Mori et al. 10737* (NY, US); Mun. Sta. Cruz de Cabralia, alrededores da Est. Ecol. Pau–Brasil, *Mori et al. 10813* (K, NY); Mun. Maraú, Faz. Agua Boa, BR 030 a 22 km a E de Ubaitaba, *Mori 12747* (NY); Rod. Lagoa Encantada, *Pinheiro 1490* (US); Mun. Santa Cruz Cabrália, area da Est. Ecol. Pau Brasil (ESPAB), ca. 16 km a W de Porto Seguro, Rod. BR 367 (Porto Seguro–Eunapólis), *Santos 73* (NY); Ilheos, *Sellow 244* (B); Mun. Belmonte, Barrolândia, Est. Exp. Gregorio Bondar, CEPLAC, 48 km E of BR 101 on rd. to Belmonte, *Thomas et al. 9926* (NY); Mun. Una, N edge of Una Biol. Res. ca. 500 m N of Rio Maruim, *Thomas et al.*

Figure 11. Lectotype of *Solanum evonymoides* (*Martius 625* [NY]).

11202 (BM). **Espírito Santo:** Mun. Linhares, Res. Flor. Porto Seguro, CVRB/BA, *Folli 1040* (NY); Mun. Rio Bananal, Faz. Santa Angelica, gated dirt rd. to rt. at 9 km from church in Bananal on paved rd. from Bananal to Novo Brasil, *Kallunki et al. 706A* (BM); Estrada X-1, Res. Linhares, Docemade, *Sucre 8298* (F); Mun. Conceição da Barra, Res. Biol. Córrego Grande (IBAMA), a 31 km de Pedro Canário (na Avenida Aracruz) e 6 km da Base do IBAMA, *Pirani et al. 3028-A* (BM, NY). **Minas Gerais:** s. loc., 1839, *Claussen s.n.* (F); Caxoeira do Campo, *Claussen 200* (G). **Rio de Janeiro:** Serra dos Orgãos, près Theresopolis, *Glaziou 8856* (G); Mun. Santa Maria de Magdalena, Par. Est. Desengano, *Martinelli 13274* (F), Pedra do Desengano, *Martinelli 13360* (F). **São Paulo:** Cunha, Res. Flor., *Custodio Filho 265* (NY); Alto da Serra, 28 Jan. 1919, *Hoehne 3042* (US); Cunha-Res., Est. de Cunha, *Martins et al. 12361* (NY).

Addition to the *Solanum amblophyllum* Species Group

Shrubs or small trees of high elevations, often growing in windswept edges of páramos; young stems and leaves glabrous to densely pubescent, the trichomes simple and uniseriate; sympodial units plurifoliate, or variously difoliate, geminate or not, if geminate, the minor leaves usually similar in size and shape to the major leaves. Leaves simple, elliptic to obovate, often thick and coriaceous. Inflorescences opposite the leaves, or occasionally lateral or appearing terminal, simple or several times branched. Flowers white, fleshy and rather large, the petals planar or campanulate at anthesis. Fruit green or yellowish, hard at maturity; fruiting pedicels deflexed or somewhat erect, woody and occasionally rugose; seeds flattened-reniform, pale tan to reddish brown.

Distribution. Species of the group occur at high elevations in the Andes, from Colombia to southern Peru.

11. Solanum elvasioides S. Knapp, sp. nov. TYPE: Ecuador. Loja: rd. Loja–Las Achiras (Urutusinga), Km 9 from the Universidad Nacional de Loja, 2800 m, 4°03′21″S, 79°13′60″W, 20 May 2001, *J. E. Madsen, A. Byg & C. Chimbo 8054* (holotype, LOJA!; isotypes, AAU!, MO!, QCA!, QCNE!). Figure 12.

Species *Solanum barbulato* Zahlbr. similis, sed caulibus et foliis ominino glabris, sympodiis unifoliatis interdum difoliatis subgeminatis, venis numerosis subparallelis differt.

Shrubs to small trees 2–5 m; stems slender, seemingly brittle, glabrous and grayish; bark pale gray on older stems; new growth glabrous or sparsely papillate, the papillae white or reddish; sympodial units usually unifoliate on reproductive shoots, occasionally difoliate and almost geminate. Leaves 3.5–9 × 0.9–2.5 cm, narrowly elliptic to lanceolate, glabrous on both surfaces, the base attenuate, the margins revolute in dry material, the apex acuminate or acute; primary veins 15 to 30 pairs, very closely spaced and subparallel, barely visible adaxially, not at all prominent abaxially, the midrib drying yellowish; petioles 0.2–0.9 cm, slightly winged from the decurrent leaf bases. Inflorescences leaf-opposed or occasionally internodal, 0.1–1 cm, simple, with 2 to 6 flowers, glabrous, the peduncle 0–0.2 cm; pedicels in flower not known, in bud glabrous; pedicel scars closely spaced and overlapping, plane with the rachis; buds (very young) elliptic. Flowers not known. Fruit a globose berry, 0.9–1 cm diam., green at maturity, glabrous; fruiting pedicels 1–1.5 cm, ca. 0.5 mm at the base, ca. 1.5 mm at the apex, deflexed to somewhat deflexed. Seeds 0.3–0.4 × 0.2–0.3 mm, flattened-reniform, reddish brown, the margins slightly paler and thickened, the surfaces minutely pitted. Chromosome number unknown.

Distribution. In páramo and shrubby vegetation from 2600–2800 m in the province of Loja, Ecuador.

Etymology. *Solanum elvasioides* is named for its leaves with tightly parallel venation that slightly resemble those of *Elvasia* DC. (Ochnaceae).

Discussion. Although *Solanum elvasioides* is only known from fruiting material, I am describing it here in order that flowering material might be sought. It must be a very rare species, as the numerous collections made in the vicinity of Loja in the last decades by collectors from the University of Aarhus and the Missouri Botanical Garden have only revealed two specimens (cited here). These collections are about 10 km apart on the western slope of the north-south ridge west of Loja (the continental divide), where the terrain drops steeply to the deep, dry Catamayo valley (D. Neill, pers. comm.).

Solanum elvasioides is superficially similar to both *S. barbulatum* Zahlbr. (of the *S. amblophyllum* Hook. species group) and *S. smithii* (of the *S. arenarium* species group), both of which occur in the same general region of southern Ecuador, but the tightly spaced, parallel leaf venation and total glabrousness of *S. elvasioides* are distinctive. It is likely to belong to the *S. amblophyllum* species group (sensu Knapp, 2002a), with other taxa from high elevations with flattened-reniform seeds. The *S. amblophyllum* species group is one of the few species groups from Knapp (2002a) that may be monophyletic (J. C. Granados-Tochoy, pers. comm.).

A preliminary conservation assessment of *Solanum elvasioides* would be Endangered (EN), based on its very small range (two currently known populations 10 km apart, so extent of occurrence < 100 km^2; see

Figure 12. Isotype of *Solanum elvasioides* (*Madsen et al. 8054* [AAU]).

IUCN, 2001) and the threat from fire spreading into its montane forest habitat from the drier forests in the heavily populated Catamayo valley (see Kessler [1992] for a discussion of threats to the forests of southwestern Ecuador).

Paratype. ECUADOR. **Loja:** Cerro Villanaco, old rd. Loja–Pedestal–La Toma, Km 10, 2670 m, 4°00′13″S, 79°15′14″W, 10 Apr. 1994, *P. M. Jørgensen, C. Ulloa & H. Vargas 44* (LOJA, MO, QCA not seen).

Additions to the *Solanum arboreum* Species Group

Shrubs or small trees; young stems and leaves glabrous to densely red-papillose, occasionally pubescent with simple, uniseriate trichomes; sympodial units difoliate and geminate, often strongly anisophyllous, occasionally unifoliate or plurifoliate. Leaves simple, narrowly elliptic to obovate, usually glabrous on both surfaces. Inflorescences opposite the leaves or occasionally internodal, simple, few to many-flowered. Flowers white, small, and with the lobes deflexed at anthesis or larger and fleshy with the lobes planar at anthesis. Fruit usually green and hard at maturity; fruiting pedicels usually erect and thickening at maturity, occasionally deflexed; seeds ovoid-reniform, pale tan to dark brown, often rather large.

Distribution. Species of the group occur in primary forests from Central America to Bolivia, at a wide range of elevations.

12. Solanum humboldtianum Granados-Tochoy & S. Knapp, Syst. Bot. 32: 202. 2007. TYPE: Colombia. Cundinamarca: Mun. Bojacá, vereda San Antonio, finca El Triunfo, saliendo hacia la carretera que conduce de Mosquera a Tena, 2600–2700 m, 29 Jan. 2005, *J. C. Granados-Tochoy & W. Meier 860* (holotype, COL!; isotypes, BM!, FMB!, HUA!).

Small trees to 10 m, diffusely branched; stems scandent, 4-angled, drying pale brownish or more yellowish when young; new growth with the vegetative buds minutely pubescent with simple, glandular 2-celled trichomes, reddish in dry material, soon deciduous; bark of older stems exfoliating; sympodial units unifoliate or difoliate, not geminate. Leaves 5–25 × 3–12 cm, elliptic to obovate, chartaceous to subcoriaceous, glabrous, markedly discolorous, adaxially bright green when fresh, becoming olive green or pale tan in dry material, somewhat shiny, abaxially yellowish green when fresh, becoming golden in dry material; base acute, slightly asymmetric; margins entire, revolute; apex acute to acuminate or emarginate, somewhat asymmetric; primary veins 7 to 14 pairs, with the midrib impressed adaxially, keeled abaxially, drying reddish green; petiole 1–4 cm, channeled adaxially, corrugated transversely and somewhat corky, glabrous. Inflorescences leaf-opposed or internodal, 1–11 cm, usually simple, occasionally bifurcate, with 6 to 50 flowers, the peduncle 0.5–5 cm, ca. 3 mm diam. at the base, stout; pedicels 0.5–1 cm, ca. 1.5 mm diam. at the base, ca. 1 mm diam. at the apex, fleshy, nodding at anthesis, glabrous, articulated at the base; pedicel scars closely spaced in 2 distinct rows, the rows and individual scars overlapping; buds globose, becoming ellipsoid, sparsely pubescent with simple trichomes to 0.5 mm, about halfway exserted from the calyx tube. Flowers heterostylous; calyx tube 2–3 mm, campanulate, the lobes 1–1.5 mm, broadly deltate to truncate, pubescent with tufts of simple, uniseriate trichomes ca. 0.5 mm at the apices, sometimes bifid and irregular; corolla 1–2 cm diam., white, fleshy, lobed ca. 2/3 of the way to the base, the lobes 0.7–1.1 cm, adaxially glabrous, abaxially densely pubescent with minute uniseriate glandular trichomes, these reddish when dry, the petal midvein keeled adaxially, the lobes cucullate at the tips; filaments with the free portion ca. 0.1 mm, the tube less than 0.2 mm, sometimes with tiny apiculae ca. 0.5 mm on the margin between the filaments, glabrous; anthers 3–4 × 1.1–5 mm, poricidal at the tips, the pores lengthening to teardrop-shaped slits with age; ovary 1–2 mm, subpyramidal, glabrous, the apex somewhat tuberculate; style straight, glabrous, short styles 2–3 mm, long styles 4–5 mm, the stigma capitate, slightly 2-lobed, the surface minutely papillate. Fruit a globose to subglobose berry, 1.5–2 × 1.2–1.6 cm, green and hard at maturity, the pericarp coriaceous; fruiting pedicels 1.5–3 cm, distally enlarged, woody and deflexed, the calyx lobes in fruit expanding to twice their size in flower, tightly investing the base of the berry; seeds ca. 30 per fruit, 3–4 × 2–3 mm, flattened-reniform, creamy yellow in fresh material, yellowish tan when dry, the margins incrassate, the surfaces minutely pitted. Chromosome number not known.

Distribution. Endemic to the western Andean slopes of Colombia in the departments of Cundinamarca and Santander, in premontane cloud forests from 2400–2700 m.

Discussion. *Solanum humboldtianum* is very similar morphologically to *S. goniocaulon* S. Knapp (of the *S. arboreum* species group) from southern Ecuador and adjacent northern Peru and to *S. laurifrons* Bitter (of the *S. amblophyllum* species group) from the Cordillera Occidental of Colombia (see Knapp, 2002a). The three species share papillate new growth,

angular fleshy stems, markedly discolorous leaves that dry yellowish abaxially, and cucullate petal apices. *Solanum humboldtianum* is also a scandent or somewhat lianescent shrub, a habit not recorded in other similar species. *Solanum goniocaulon* is a member of the *S. arboreum* species group (sensu Knapp, 2002a), all with ovoid seeds. Within the *S. arboreum* species group, it may be that *S. humboldtianum* is closely related, as well as morphologically similar, to *S. goniocaulon*.

Solanum humboldtianum has been assessed as Endangered (EN) using IUCN Red List criteria (IUCN, 2001), based on its extent of occurrence of less than 5000 km^2, area of occupancy less than 100 km^2, its severely fragmented (four current locations) and small overall population size, and the unprotected nature of most of the populations near a major population center (Granados-Tochoy et al., 2007). *Solanum humboldtianum* is being propagated in the Jardín Botánico de Bogotá José Celestino Mutis, with plants crossing easily and producing ample seed (J. C. Granados-Tochoy, unpubl.), making ex situ conservation a possibility.

Additional specimens examined. COLOMBIA. **Cundinamarca:** Mun. Bojacá, vereda San Antonio, *Granados-Tochoy et al. 389* (COL), *Granados-Tochoy & Giraldo-Cañas 844* (COL); near Sibaté, San Miguel, *Hawkes & García-Barriga 36* (K); Santa Fé de Bogotá, 1805, *Humboldt & Bonpland s.n.* (P); Mun. Granada, hac. "El Soche," *Idrobo 5437* (COL, FMB); Mun. Bojacá, vereda San Antonio, "La Merced," próximo a la carretera Mosquera–Tena, *Lozano-C. & Torres-R. 98* (COL); Mun. Cabrera, vereda Núñez, *Morales et al. 497* (COL). **Santander:** Mun. El Encino, vereda Santa Helena, predio La Sierra, margen izquierdo aguas abajo del Río La Rusia, Santuario de Flora y Fauna Guanetá, Alto Río Fonce, *Cadena et al. 78* (COL, UIS).

13. Solanum sagittantherum Granados-Tochoy & C. I. Orozco, Caldasia 28(1): 2. 2006. TYPE: Colombia. Cundinamarca: Mun. Sibaté, sector El Soche, vía Bogotá–Silvania, ca. 2900 m, 31 May 2005, *J. C. Granados-Tochoy, D. Giraldo-Cañas & D. Canal 888* (holotype, COL!; isotypes, FMB not seen, HUA not seen).

Subshrub to 1.5 m, rhizomatous; young stems slightly angular, minutely papillose with trichomes less than 0.05 mm, sparsely pubescent with simple uniseriate trichomes to 0.5 mm, glabrescent; new growth papillose, puberulent, the trichomes to 0.5 mm, leaf margins in bud ciliate distally; sympodial units difoliate, geminate, anisophyllous. Leaves elliptic to ovate, occasionally obovate, chartaceous, larger on vegetative shoots, adaxially dark green in fresh material, drying olive green to brownish, abaxially yellowish, drying golden-green, glabrous on both surfaces, abaxially minutely puberulent on the veins, the trichomes simple, uniseriate, to 0.5 mm, the veins pale green in fresh material, drying brownish green; major leaves 3–21 × 1–8 cm, the base acute to cuneate, occasionally oblique, the margins entire to slightly sinuate, revolute when dry, the apex acute to acuminate, inconspicuously ciliate at the tip; minor leaves differing from the major leaves only in size, 0.5–3 × 0.3–2 cm; primary veins 4 to 12 pairs, impressed to slightly prominent adaxially, prominent abaxially; petioles 0.2–3 cm, glabrous, channeled adaxially, with a few transverse stripes near the base. Inflorescences opposite the leaves, 0.5–0.9 cm, simple, with 3 to 8 flowers, papillose and sparsely pubescent like the young stems, the peduncle 0.05–0.5 cm; pedicels at anthesis 0.5–1 cm, weakly to strongly reflexed, somewhat enlarged at the apex, articulated at the base; pedicel scars closely packed, somewhat separated or overlapping; buds globose when young, later ellipsoid, one calyx lobe prominently veined and larger than the rest, the corolla strongly exserted from the calyx tube. Flowers tetramerous, isostylous; calyx white or greenish white in fresh material, cream in dry material, the tube ca. 1 mm, broadly conic, glabrous or pubescent on external surface with simple uniseriate trichomes ca. 0.5 mm, on internal surface minutely glandular-papillose, the papillae less than 0.1 mm, the lobes 1–1.5 × 1.5–3 mm, broadly deltate, glabrous or sparsely pubescent with simple uniseriate trichomes and glandular papillae like the tube, the apex of one of the lobes often enlarged to a fleshy projection ca. 0.2 mm, an extension of the midvein; corolla 1.5–2 cm diam., fleshy, white, stellate, lobed 2/3 of the way to the base, the lobes 0.5–0.9 cm, elliptic, planar at anthesis, cucullate at the tips, the tips and margins minutely papillate, the papillae less than 0.1 mm; filaments with the free portion ca. 0.1 mm, the tube 1.5–2 mm, glabrous; anthers 4–5 × ca. 1 mm, sagittate at the base, poricidal at the tips, the pores lengthening to slits with age; ovary ca. 1 × 1 mm, conical, glabrous; style 6–8 mm, straight or sigmoidally curved, white in live plants, the stigma ca. 1 mm diam., capitate, the surface minutely papillate. Fruit a globose berry, 1–1.5 × 1–1.5 cm, green at maturity, drying brownish green; fruiting pedicels 1.5–2 cm, strongly deflexed beneath the foliage, distally enlarged, the calyx lobes in fruit ca. 2 times the size at anthesis, tightly appressed to berry in fresh material, reflexed in dry material; seeds ca. 2–3 mm, flattened-reniform, pale green in fresh material, reddish brown in dry material, the margins enlarged, the testa minutely pitted. Chromosome number not known.

Distribution. Only known from the type locality on the western slopes of the Cordillera Oriental of the

Colombian Andes, in pristine cloud forest from 2700–2900 m.

Discussion. *Solanum sagittantherum* is unusual in the Geminata clade in possessing tetramerous flowers, but other species originally described with tetramerous flowers have later been shown to have populations with pentamerous flowers as well (e.g., *S. cruciferum* Bitter, see Knapp, 2002a). Should more populations of *S. sagittantherum* be found, they may indeed prove to be pentamerous. Granados-Tochoy and Orozco (2006) were uncertain of the affinities of *S. sagittantherum*, as it combines many of the characteristics of several of the pragmatic species groups delimited by Knapp (2002a). Its flattened seeds and apically enlarged fruiting pedicel make it similar to members of the *S. leucocarpon* group, while the rhizomatous habit is shared with both *S. arboreum* and *S. robustifrons* Bitter (of the *S. arboreum* and *S. robustifrons* species groups, respectively). The rhizomatous habit is unusual in the Geminata clade and may be related to the humid nature of the habitat in which *S. sagittantherum* occurs. The sagittate anther bases for which the species is named are also very unusual in the Geminata clade, but are also present in *S. amblophyllum* of central Peru, which differs from *S. sagittantherum* in distribution, in having plurifoliate, rather than difoliate and geminate, sympodial units, and in its complex branched inflorescences, and in *S. chalmersii* of Bolivia, which is densely pubescent. I have tentatively placed *S. sagittantherum* in the *S. arboreum* species group based on its overall morphology, but molecular results may clarify its status.

Solanum sagittantherum is known only from the type locality (four collections were cited in Granados-Tochoy and Orozco [2006], all from El Soche, but no population details were reported; I have not seen this material) and needs further investigation before a confident conservation status can be assigned. Given the fragmented nature of the cloud forest habitat in the Colombian Department of Cundinamarca (Granados-Tochoy et al., 2006), it is likely to be of conservation concern, but it can be given a preliminary assessment (IUCN, 2001) of Critically Endangered (CR) due to its very restricted distribution (< 100 km^2) in a single locality.

Addition to the *Solanum nigricans* Species Group

Shrubs or small trees; young stems and leaves usually pubescent with usually arachnoid, but occasionally dendritic or simple trichomes; sympodial units unifoliate, difoliate or trifoliate, or difoliate and geminate. Leaves simple, elliptic to ovate, usually pubescent abaxially, occasionally glabrous, the trichomes like those of the young stems. Inflorescences opposite the leaves, 3- to 20-flowered. Flowers white, thick and fleshy, the corolla lobes planar at anthesis; ovary glabrous or pubescent. Fruit green and hard at maturity, glabrous or pubescent; fruiting pedicels erect or somewhat deflexed, woody; seeds ovoid-reniform, usually dark reddish brown.

Distribution. Species of the group are usually found in forests, in montane forests from Mexico to Honduras and the Andes, and in *Araucaria* and coastal forests in southeastern Brazil.

14. Solanum canoasense L. B. Sm. & Downs, Fl. Ilustr. Catarin., Pt. 1 Solanac. 104. 1966. *Solanum cataractae* L. B. Sm. & Downs, Phytologia 10: 427. 1964. TYPE: Brazil. Santa Catarina: Mun. Bom Retiro, Rio Canoas, Campo dos Padres, 1300–1400 m, 22 Nov. 1956, *L. B. Smith & R. Klein 7843* (holotype, US 00026997!; isotypes, HBR not seen, R not seen).

Small shrubs 0.5–2 m; stems slender, erect, glabrescent; bark reddish; new growth densely pubescent with uniseriate dendritic and/or arachnoid trichomes, these soon deciduous; sympodial units plurifoliate. Leaves 3–9 × 1–2.1 cm, lanceolate to oblanceolate to narrowly lanceolate or oblanceolate, widest in the upper 1/3, both surfaces glabrous, the leaves crowded at branch apices, the base attenuate, the margins revolute, the apex acute; primary veins 7 to 9 pairs, drying paler than the lamina; petioles 0.4–0.6 cm, sparsely dendritic pubescent adaxially. Inflorescences internodal, appearing terminal, 0.6–1 cm, simple, with 2 to 4 flowers, densely pubescent with uniseriate arachnoid or dendritic trichomes, the peduncle 0.2–0.5 cm; pedicels 1.3–1.6 cm, ca. 1 mm diam. at the base, ca. 1.5 mm diam. at the apex, stout, ± nodding at anthesis, sparsely branched pubescent like the inflorescence, articulated at the base; pedicel scars 1–2 mm apart; buds ellipsoid, the corolla strongly exserted from the calyx tube. Flowers all perfect (?); calyx tube (1–)2–2.5 mm, broadly conical, the lobes 2–4 mm, broadly and irregularly deltate, thick and fleshy, the margins thickened and paler when dry, sparsely pubescent with arachnoid or dendritic trichomes especially near the tips; corolla 2–2.2 cm diam., white, lobed ca. 2/3 of the way to the base, the lobes 1–2 cm, broadly deltate, ± planar at anthesis, sparsely to densely pubescent abaxially especially in the sinuses and distally with branched uniseriate or papillate trichomes, these especially dense at the tips; filaments with the free portion 1–1.5 mm, the tube ca. 1 mm, glabrous; anthers 5–6 × 1.5–2 mm, poricidal at the tips, the pores lengthening to slits with age; ovary conical, glabrous, the style 1–1.1 cm, straight, glabrous, the stigma clavate, the

surface minutely papillate. Fruit not seen; seeds not seen. Chromosome number not known.

Distribution. In *Araucaria* forests, cloud forests, and forest edges in the Brazilian states of Santa Catarina and Paraná, from 1300–1400 m.

Common name. "Canema mirim" (Smith & Downs, 1966).

Discussion. Solanum canoasense is distinctive among southeastern Brazilian members of the Geminata clade in its large flowers and floccose, but soon glabrescent, new growth. The species is known from very few collections, and it is apparently very rare. Mentz and Oliveira (2004) put *S. canoasense* in synonymy with *S. cassioides* L. B. Sm. & Downs, from which it differs in not possessing the distinctive broad-based trichomes found in *S. cassioides* and its relative *S. trachytrichium* Bitter, and in its plurifoliate, rather than difoliate, sympodial units. *Solanum canoasense* is a member of the *S. nigricans* M. Martens & Galeotti species group (sensu Knapp, 2002a) based on the possession of floccose trichomes on the new growth, but seed morphology is not known. The type of *S. canoasense* is nearly glabrous, like *S. bahianum* S. Knapp of coastal Bahia, Brazil. *Solanum canoasense* differs from *S. bahianum* in its plurifoliate rather than unifoliate sympodia, and in its larger flowers that are somewhat campanulate at anthesis. The two species occupy very different habitats, *S. bahianum* the coastal restingas and *S. canoasense* upland *Araucaria* forests. No fruiting specimens are known, and collections of fruiting material are a priority.

Conservation assessments of *S. canoasense* are difficult, due to the few specimens available. The small area of occupancy (few collections) and restricted distribution of the species in threatened habitat (*Araucaria* forests) suggest it is of concern and could be assigned a preliminary conservation status (IUCN, 2001) of Endangered (EN).

Additional specimens examined. BRAZIL. **Paraná:** Mun. Guaratuba, Serra do Araçatuba, *Kummrow 2410* (NY). **Santa Catarina:** Mun. Urubici, estrada Serra do Corvo Branco, *Stehmann 1756* (NY).

Additions to the *Solanum arenarium* Species Group

Small shrubs; young stems and leaves sparsely to densely pubescent with dendritic trichomes, these often very densely branched; sympodial units unifoliate or more commonly difoliate and geminate. Leaves simple, lanceolate to elliptic, occasionally shiny adaxially, pubescent abaxially with dendritic trichomes, these confined to the vein axils or densely covering the surface, the apex and base various. Inflorescences opposite the leaves or occasionally internodal, simple, densely pubescent like the stems and leaves, the trichomes dendritic; pedicel scars closely spaced or unevenly spaced and ca. 5 mm apart. Flowers white, usually somewhat fleshy, the corolla lobes planar at anthesis. Fruit green or greenish yellow and hard at maturity, the pericarp occasionally thin and brittle in dry specimens, glabrous or densely pubescent with dendritic trichomes; fruiting pedicels woody, deflexed; seeds very large, ovoid-reniform, pale tan or yellow.

Distribution. Species in the group are distributed in montane forests in the Andes in Ecuador, Peru, and Bolivia, and in a variety of habitats in southeastern Brazil.

15. Solanum compressum L. B. Sm. & Downs, Phytologia 10: 430. 1964. TYPE: Brazil. Santa Catarina: São Joaquim, Fazenda de Laranja. Bom Jardim, 1400 m, 13 Dec. 1958, *P. Reitz & R. Klein 7867* (holotype, US 00027001!; isotypes, HBR not seen, NY!). Figure 13.

Trees to small treelets 5–10 m, to 15 cm DBH; stems spreading, sparsely pubescent with loose, golden uniseriate dendritic trichomes 0.5–1 mm, soon glabrescent; bark dark reddish brown; new growth densely golden dendritic pubescent, the trichomes ca. 1 mm; sympodial units plurifoliate. Leaves 4–15 × 1.6–5 cm, elliptic to narrowly elliptic, glabrous and slightly shiny adaxially, occasionally with a few scattered uniseriate simple trichomes on the lamina, abaxially very variably pubescent, from a few denditic trichomes 0.5–1 mm in the vein axils to sparsely pubescent over the entire lamina with dendritic trichomes 0.5–1 mm, the trichomes with a uniseriate stalk and very short branches, always denser in the vein axils, the base acute to attenuate, the margins entire, the apex acute; primary veins 9 to 10 pairs, the midrib keeled adaxially; petioles 1–2.5 cm, glabrous or sparsely dendritic pubescent. Inflorescences terminal, much longer than the new growth, 4–9 cm, 3 to 4(to 6) times branched, with 30 to 40 flowers, glabrous or sparsely pubescent with loose dendritic trichomes like those of the stems, the peduncle 2–6 cm; pedicels 1.4–1.6 cm, ca. 0.5 mm diam. at the base, ca. 1 mm diam. at the apex, filiform, ± deflexed at anthesis, glabrous to sparsely dendritic pubescent; pedicel scars irregularly spaced 0.5–1 mm apart, plane with the rachis; buds ellipsoid, pointed at the tips, strongly exserted from the calyx tube. Flowers apparently all perfect; calyx tube 1–1.5 mm, broadly conical, the lobes 1.5–2.5 mm, deltate, densely pubescent with dendritic trichomes ca. 0.5 mm on the tips; corolla 1.5–2 cm diam., white, lobed ca. 3/4 of the way to the

Figure 13. Isotype of *Solanum compressum* (*Reitz & Klein 7867* [NY]).

base, the lobes 1–1.5 cm, deltate, planar at anthesis, minutely dendritic pubescent on the tips and margins; filaments with the free portion ca. 0.5 mm, the tube ca. 0.5 mm or less, glabrous; anthers 3.5–4 × 1.5–2 mm, poridical at the tips, the pores lengthening to slits with age, the abaxial surface papillate and rough; ovary conical, glabrous, the style 6–7 mm, straight, glabrous, the stigma clavate, 2-lobed, the surface minutely papillate. Fruit a globose berry, 1–1.5 cm diam., pale green at maturity, the pericarp woody when dry; fruiting pedicels 2.5–3 cm, ca. 1.5 mm diam. at the base, ca. 3 mm diam. at the apex, pendent, the calyx lobes enlarging in fruit to 3–4 mm; seeds ca. 2 × 1.5 mm (immature?), ovoid-reniform, pale yellow, the surfaces minutely pitted, the margins not incrassate. Chromosome number not known.

Distribution. In Brazil (Rio Grande do Sul, Santa Catarina, and Paraná) and adjacent Argentina and Paraguay, in open forests on the planalto, interior Atlantic Forest, and *Araucaria* forests, from 750–1400 m.

Common name. "Canema mirim" (Smith & Downs, 1966).

Discussion. Solanum compressum is somewhat similar to *S. pabstii*, also of southeastern Brazil, but the dendritic pubescence, larger flowers, and more robust inflorescences easily distinguish it from that species. The trichomes and ovoid seeds of *S. compressum* place it as a member of the *S. arenarium* species group (sensu Knapp, 2002a) and are particularly like those of *S. gnaphalocarpon* Vell. from Brazil. *Solanum gnaphalocarpon* has difoliate, geminate sympodia that are anisophyllous, a simple inflorescence, and a distinctive densely long-pubescent fruit. The seed morphology of *S. compressum* needs further examination, as those I have examined are from what may be immature fruits.

Solanum compressum is very variable in pubescence density throughout its range, with some plants being nearly glabrous and others densely pubescent on all parts. The trichomes are always dendritic, with a long uniseriate stalk and very short branches. The abaxial surface of the anthers of *S. compressum* is papillate in herbarium specimens and somewhat reminiscent of the anther margins of wild tomatoes (Carrizo García, 2003; Peralta et al., 2008). Whether this is merely an artifact of drying is not known, but it occurs in all flowering specimens I have examined.

Solanum compressum is relatively widespread in the Atlantic Interior Forest of Brazil, Argentina, and Paraguay. It grows often in disturbed habitats at the edges of forests and roads. It can be assigned a preliminary conservation status of Near Threatened (NT) (IUCN, 2001), due to the fact that the habitat in which it occurs (Atlantic Interior Forest or Selva Paranaënse) is highly threatened; monitoring will be necessary.

Additional specimens examined. ARGENTINA. **Misiones:** Dpto. Gral. Manuel Belgrano, Ruta Nac. 101, 8 km de Bernardo de Irigoyen hacia San Antonio, Salto Andrecito, *Morrone et al. 1388* (MO [2]); Guaraní, Predio Guaraní, junto a la casa de guardaparques, *Tressens et al. 5910* (F, NY, US). BRAZIL. **Paraná:** Mun. Guarapuava, Serra da Esperança, *Hatschbach 7384* (F); Mun. Laranjeiras do Sul, Rio das Pedras, *Hatschbach & Pereira 13071* (US); Vittorino, *Hatschbach 22679* (BM, F, W); Mun. Gal. Carneiro, Passo da Galinha, *Hatschbach 30713* (BM, NY); Itaperussú, *Jonsson 1005a* (F); Fazenda Reserva, ca. 60 km SW of Guarapuava, *Lindeman & de Haas 4915* (K [2], NY). **Rio Grande do Sul:** Tres Arroios, S. Almeida, BR 153, *Butzke et al. 7468* (US); Mun. São Marcos, Linha Feijó, *Kegler 286* (NY, US); Mun. Caixas do Sul, Forqueta, *Kegler 1301* (US); Mun. Caixas do Sul, Vila Oliva, *Kegler 1325* (US); São Francisco de Paula, no paradeiro da estrada para Taquara, *Mentz & Sobral 215* (NY); sentre o rio Tainhas e Princesa do Campo, *Pabst 6693 & Pereira* (F, NY, US); Vila Oliva p. Caixas, *Rambo 44621* (W); Kappesberg, p. Montenegro, *Rambo 52193* (US [2]); São Francisco de Paula, Carapina, *Rossato et al. 2484* (G, US); Morro Zeuter, Dois Ermãos, *Sehnem 12505* (US); Linha Imperial, Nova Petrópolis, *Sobral et al. 7991* (F); Par. de Espigão Alto, Barrancão, *Stehmann 728* (NY); Barra do Ouro, estrada para Riozinho, *Stehmann et al. 1524* (NY); Cambará, *Stehmann ICN 111359* (NY); Faxinal, Cambará do Sul, *Stehmann & Sobral ICN 111362* (NY); Mun. São Francisco de Paula, estrada para Taquara, *Wasum 1174* (G, US), *Wasum 1422* (US); Caixas do Sul, Jardim Botânico, *Wasum et al. 9015* (G, US). **Santa Catarina:** Faz. Laranja, Bom Jardim, S. Joaquim, *Reitz & Klein 7867* (NY); Urupema, São Joaquim, *Reitz & Klein 14584* (US); Rio Lavatudo, São Joaquim, *Reitz & Klein 15947* (US); Potreiro Grande, Mafra, *Reitz & Klein 17422* (F). PARAGUAY. **Itapuá:** Pirapó, Centro de Desarrollo Forestal, *Pérez 173* (MO).

16. Solanum pseudodaphnopsis L. A. Mentz & Stehmann, Novon 13: 97. 2003. TYPE: Brazil. Paraná: Paranaguá, Matinhos, 20 Sep. 1946, *G. Hatschbach 382* (holotype, MBM not seen; isotypes, BM!, PACA not seen, US 00810597!, US 00810597!, W!).

Shrubs to 1.7 m; branches woody, cylindrical, dark green to almost dark brown, glabrate, apical branches light, tinged in green or gold; new growth sparsely to densely covered with dendritic-echinoid trichomes; sympodial units unifoliate, less commonly difoliate and geminate. Leaves 5–12.5 × 1.5–5.5 cm, obovate, coriaceous and shiny, drying olive green to cinereous, glabrous or with a few dendritic-echinoid trichomes adaxially along the veins, glabrous or with a few dendritic-echinoid trichomes along the midrib abaxially, the base attenuate, the margins slightly revolute in dry material, the apex obtuse, rounded or slightly acute; primary veins 4 to 7 pairs, drying yellow abaxially; petiole 0.3–0.5 cm, sparsely pubescent

with dendritic-echinoid trichomes. Inflorescences opposite the leaves or occasionally internodal, simple or rarely branched, 1–4 cm, 12- to 19-flowered, the peduncle to 2 cm, entire inflorescence pubescent with dendritic-echinoid trichomes; pedicels at anthesis slender, to 2 cm, sparsely pubescent with dendritic-echinoid trichomes, articulated at the base; pedicel scars ca. 1 mm apart, not overlapping, beginning 1/3 to 1/2 of the way from the base of the inflorescence; buds ellipsoid, the corolla soon exserted from the calyx tube. Flowers apparently all perfect; calyx tube conical, ca. 2 mm long, the lobes ca. 2 mm, ovate, sparsely dendritic-echinoid pubescent adaxially, glabrous abaxially; corolla 1.5–2 cm diam., white, lobed ca. 3/4 of the way to the base, the lobes reflexed at anthesis, glabrous or with scattered dendritic-echinoid trichomes adaxially, the tips of the lobes with a few dendritic-echinoid trichomes, otherwise papillate; anthers 2–3 × ca. 0.5 mm, poricidal at the tips, the pores teardrop-shaped; free portion of the filaments less than 0.1 mm long, the filament tube ca. 0.5 mm; ovary glabrous; style to 7 mm; stigma a thickened area at the tip of the style, minutely papillose. Fruit a globose, glabrous berry, to 1 cm diam., green; fruiting pedicels woody, somewhat deflexed, to 2.5 cm long, ca. 1 mm diam. at the base, ca. 2.5 mm diam. at the apex; seeds ca. 3 × 2.5 mm, ovoid-reniform, brown, the surfaces minutely pitted, nearly smooth.

Distribution. Restricted to the coastal sandy habitats of restinga vegetation in Santa Catarina and Paraná in southern Brazil; growing in open places and in gaps along the borders of coastal rain or swamp forest at about sea level.

Common names. Brazil. Paraná: "canema-mirim, caneminha, canema do brejo" (*Hatschbach 382*).

Discussion. Solanum pseudodaphnopsis is easily recognized by its obovate, leathery, glabrous, or very sparsely pubescent leaves with delicate, dendritic-echinoid trichomes. The leaves dry a distinctive olive-green color. The trichome branches of *S. pseudodaphnopsis* are not as congested as the true echinoid trichomes found in members of the Brevantherum clade (e.g., *S. erianthum* D. Don or *S. rugosum* Dunal) or in the *S. nitidum* Ruiz & Pav. species group of the Dulcamaroid clade (e.g., *S. storkii* C. V. Morton & Standl.), but the branches are more tightly packed along the axis than other related species in the Geminata clade (sensu Bohs, 2005). *Solanum pseudodaphnopsis* is a member of the *S. arenarium* species group and within that group is most similar to *S. arenarium*, differing in its larger, obovate leaves and much sparser, somewhat echinoid trichomes. *Solanum pseudodaphnopsis* also is morphologically similar to *S. cassioides* of the *S. nudum* species group, but differs from that species in its leaf shape, ovoid-reniform seeds, and simple inflorescences; within the *S. nudum* species group, *S. cassioides* is related to *S. trachytrichium*. Both species possess peculiar thick-based trichomes not present in *S. arenarium* and *S. pseudodaphnopsis*. *Solanum arenarium* is a species of upland forests in southeastern Brazil, mainly on granitic hills, at elevations to ca. 300 m, while *S. pseudodaphnopsis* is restricted to the restinga habitat at sea level.

Solanum pseudodaphnopsis occurs in a very restricted and threatened habitat type, the coastal restinga of Brazil (see Mentz & Stehmann, 2003). It is known from very few (10 cited in the original description) collections along a narrow, ca. 100–150 km long, coastal strip of critically endangered habitat (Falkenberg, 1999). Based on these observations, it can be assigned a preliminary conservation status of Endangered (EN) using IUCN criteria (IUCN, 2001), but may indeed turn out to be Critically Endangered (CR) when detailed population data become available.

Additional specimens examined. BRAZIL. **Paraná:** Guaratuba, Rio da Praia, *Hatschbach 10198* (US); Mun. Paranaguá, Rio da Vila, *Hatschbach 43175* (F).

Addition to the *Solanum dolosum* Species Group

Spindly shrubs or hemiepiphytes; young stems and leaves variously pubescent, strongly zigzag; sympodial units unifoliate. Leaves simple, linear to ovate, glabrous to variously pubescent, the apex long-acuminate, the margins of the apex with minute ciliate trichomes. Inflorescences opposite the leaves or internodal, strongly zigzag. Flowers white, usually less than 1 cm diam., the lobes reflexed at anthesis. Fruit a globose green berry; fruiting pedicels deflexed; seeds ovoid-reniform.

Distribution. Species of the group occur in montane Andean cloud forests from Colombia to Bolivia.

17. **Solanum naucinum** S. Knapp, Ann. Missouri Bot. Gard. 92: 251. 2005. TYPE: Peru. Pasco: Prov. Oxapampa, 4–5 km N of Mallampampa, 2400 m, 10°02′S, 75°45′W, 22 Jan. 1984, *Smith & Canne 5793* (holotype, USM!; isotype, MO!).

Small shrub to 50 cm; young stems and leaves densely golden pubescent, the trichomes 0.5–1 mm, dendritic, uniseriate with single-celled branches; older stems glabrescent, thin and flexuous; bark of the older stems pale brown; sympodial units unifoliate, occasionally difoliate and geminate on non-

reproductive nodes. Leaves 7–15 × 1.5–3.2 cm, narrowly elliptic to narrowly oblanceolate or lanceolate, glabrous adaxially, pubescent abaxially on the veins and lamina with golden uniseriate dendritic trichomes to 1.5 mm, the trichome branches unicellular, pubescence denser along the veins, the apex long-acuminate, blunt at the extreme tip, the margins entire, slightly revolute, the base acute, sometimes somewhat oblique; primary veins 8 to 11 pairs, these looping to form a distinct marginal vein; petiole 0.3–0.6 cm, densely pubescent; minor leaves on non-reproductive nodes smaller, but similar in shape to the major leaves. Inflorescences opposite the leaves, simple, 5–8 cm, 8- to 10-flowered, densely pubescent with golden dendritic trichomes to 1 mm; pedicels at anthesis not seen, articulated at the base; pedicel scars spaced 2–4 mm apart, beginning ca. 1 cm from base of inflorescence; buds not seen. Flowers white (fide *Smith & Canne 5793*), not seen. Fruit a globose, green (immature?) berry, ca. 1 cm diam., drying mustard yellow on herbarium specimens; fruiting pedicels 1–1.2 cm, pendent, ca. 1 mm diam. at the base, ca. 2 mm diam. at the apex; calyx lobes in fruit ca. 3 mm, deltate, pubescent with golden dendritic trichomes; seeds ca. 3 × 2 mm, not mature in fruit examined, ovoid-reniform to slightly flattened, the surfaces minutely pitted, the margins not incrassate.

Distribution. Only known from the type specimen collected in the montane forests of the Cordillera de Yanachaga (site of Parque Nacional Yanachaga-Chemillén) in the Department of Pasco, Peru, at about 2400 m.

Discussion. *Solanum naucinum* is a member of the *Solanum dolosum* C. V. Morton ex S. Knapp species group (sensu Knapp, 2002a), with unifoliate sympodia on reproductive nodes, filamentous inflorescences, and long-acuminate leaves with ciliate margins near the apex. It differs from all other species in the group in its distinctive golden dendritic pubescence. Like the other taxa in the *S. dolosum* species group, *S. naucinum* is a spindly shrub, possibly also growing as a hemiepiphyte in its cloud forest habitat. The seeds in the fruits on the type specimen are immature, but appear to be ovoid-reniform without incrassate margins; however, mature fruits and flowers are needed to complete the morphological description of this rare species. *Solanum naucinum* is apparently an extremely rare plant; no new collections of this rare species have come to light since the collection of the type specimen in 1984. *Solanum naucinum* shares an elongate inflorescence with widely spaced pedicel scars with *S. gonyrhachis* S. Knapp of Bolivia.

Solanum naucinum is of uncertain conservation status (IUCN, 2001) as it is known only from the type, and our knowledge is limited due to lack of collections. It has been given the status of Data Deficient (DD) (Knapp et al., 2007), but it is likely to be Endangered (EN) or Critically Endangered (CR) due to low population size (common in other members of the *S. dolosum* species group) and very restricted range (Knapp, 2005).

Species Incertae Sedis

18. Solanum sumacaspi S. Knapp, Ann. Missouri Bot. Gard. 92: 248. 2005. TYPE: Peru. Cusco: Urubamba, rd. from Urubamba to Quillabamba, betw. Ollantaytambo & Abra Malaga, 13°12′15″S, 72°17′49″W, ca. 3500 m, 12 Sep. 2002, *M. Ackermann & D. Kollehn 289* (holotype, USM!; isotypes, BM!, BSB!, HUSA not seen, M!, NY!).

Small shrubs to 2 m tall; young stems and leaves minutely and sparsely papillate, soon completely glabrous, pale greenish yellow and shiny, a few (at most 1 or 2) simple uniseriate trichomes sometimes present on very new stems; older stems ridged, shiny; bark of the older stems and trunks pale greenish white, exfoliating; sympodial units difoliate, apparently geminate, the inflorescences borne at the branch tips and overtopping the leaves. Leaves simple, elliptic, completely glabrous adaxially and abaxially, shiny, thick and fleshy, drying olive green adaxially, pale bright yellowish green abaxially; major leaves 3–11 × 1.3–5 cm, the apex acute to somewhat truncate, the base attenuate onto the petiole; primary veins 5 to 10 pairs, raised and yellowish green adaxially, prominent and bright yellow abaxially; petioles 0.1–1 cm, ridged, the base remaining as a prominent scar on the older stems; minor leaves not differing from the major ones in size or shape. Inflorescences opposite the leaves, simple, 1–2 cm, 7- to 10-flowered, glabrous and shiny, the peduncle 0.5–1 cm; pedicels at anthesis somewhat deflexed, 0.5–1 cm long, tapering from the abrupt base of the calyx tube to a slender base 0.5–0.8 mm diam., green and shiny, articulated at the base; pedicel scars widely spaced ca. 2 mm apart, the pair of scars closer than the distance between the scar pairs, flowers borne from near the base of the inflorescence; buds when very young appearing globose, the corolla soon exserted from the calyx lobes, the buds later becoming ellipsoid just before anthesis. Flowers all apparently perfect; calyx tube 1.5–2 mm, broadly open and conical, the lobes 2–2.5 mm, deltate to rounded, glabrous, the margins thickened and drying white, the tip with a thickened mucro ca. 1 mm which may be swollen in fresh material, occasionally notched; corolla white or cream-colored, 1.4–2.5 cm diam.,

lobed nearly to the base, the lobes planar or somewhat campanulate at anthesis, glabrous on both surfaces, the tips of the lobes minutely papillose and somewhat cucullate; filaments with the free portion ca. 1 mm long, the tube ca. 0.25 mm long to absent; anthers 5–6 × 1–1.5(–2) mm, poricidal at the tips, the pores teardrop-shaped, the tips thickened and paler; ovary glabrous; style straight, 1–1.2 mm; stigma capitate, green. Fruit and seeds not known.

Distribution. In high secondary forests near steppeland in southern Peru, from 2100–3400 m, with most collections from the vicinity of Cusco.

Discussion. *Solanum sumacaspi* was included in the delimitation of *S. daphnophyllum* Bitter, a similarly glabrous species from Bolivia in the *S. nudum* species group, with some reservations in the monograph of section *Geminata* (Knapp, 2002a). Additional collections confirmed the distinctness of *S. sumacaspi*, both morphologically and in habitat. *Solanum daphnophyllum* is a species of middle to high elevation (700–2800 m), dry to semi-humid forests on the eastern Andean slopes from central Bolivia to the Department of Puno in Peru, while *S. sumacaspi* is only found in disturbed forests in much higher elevations (2100–3500 m) in grassland (puna)/forest transition areas in the Urubamba River drainage. Morphologically, *S. sumacaspi* differs from *S. daphnophyllum* in its more elongate inflorescences with more widely spaced pedicel scars and in its pale green, exfoliating bark. *Solanum daphnophyllum* has inflorescences usually only ca. 1 cm long and dark reddish brown shiny bark on all stems. The apparently geminate sympodial units also differentiate *S. sumacaspi* from *S. daphnophyllum*, which has difoliate, but not geminate, units.

Fruiting material is lacking for *Solanum sumacaspi*, preventing its firm allocation to one of the putative species groups of the Geminata clade (section *Geminata*, sensu Knapp, 2002a). Should the seeds be flattened-reniform, *S. sumacaspi* is likely to be a member of the *S. nudum* species group (sensu Knapp, 2002a), along with *S. daphnophyllum*. It differs from other completely glabrous members of the *S. nudum* species group in its unwinged pedicels, deltate calyx lobes, and high elevational distribution.

Solanum sumacaspi also morphologically resembles two species in the *S. arboreum* species group (sensu Knapp, 2002a) in its showy inflorescences of flowers all apparently opening at once: *S. gratum* Bitter of the Cordillera de la Costa in Venezuela and *S. plowmanii* S. Knapp of the western Andean slopes in Peru and Ecuador. The shiny stems also are similar to *S. goniocaulon* of the eastern Andean slopes of northern Peru and southern Ecuador, also of the *S. arboreum* species group. It can be differentiated from all those species by the combination of its yellowish exfoliating bark, shiny leaves that dry a very pale green, its non-anisophyllous leaves, and its high-elevation southern Peruvian distribution.

Solanum sumacaspi appears to be relatively common in the narrow Cusco area. It can tentatively be assigned a conservative preliminary conservation status of Endangered (EN) based on IUCN criteria (IUCN, 2001) of range (< 5000 km^2), number of locations (< 5 to 6), and habitat disturbance; the species was treated as Data Deficient (DD) by Knapp et al. (2007). Further investigations at the population level, however, may reveal a more significant threat status. Label data indicate the species grows in disturbed forests, but this may be indicative of the highly altered state of the habitat in the region rather than indicative of the weedy status of *S. sumacaspi*.

Additional specimens examined. PERU. **Cusco:** Urubamba, Ollantaytambo, Huaytampo, *Calatayud et al. 966* (BM, MO, NY, USM), *Farfán et al. 373* (BM, CUZ not seen, HUT not seen, MO, NY, USM not seen); Urubamba, Yucay, Huaran, *Farfán & Tupayachi 424* (MO, NY); Urubamba, Macchu Picchu, above town of Pauqarcancha, *Peyton & Peyton 147* (MO); Urubamba, Macchu Picchu, along Inca trail in Tres Piedras Blancas, 1 km from Huayabamba, *Peyton & Peyton 1026, 1026b* (MO); Calca, Lares, Manto, *Valenzuela et al. 1214* (MO, NY).

Revised Key to the Species of the Geminata Clade

Dichotomous key to the species of the Geminata clade

In contrast to the keys in Knapp (2002a), here I include all the species of the Geminata clade in a single key, rather than breaking them up into the pragmatic species groups defined in that monograph. The key includes only species found in the New World; if you are in the Old World and encounter a species of the Geminata clade, it is *Solanum spirale* with red fruits on deflexed pedicels or the introduced *S. diphyllum* L. with orange fruits on erect pedicels (see Knapp, 2002a). I have tried to use vegetative characters as much as possible, largely because many of these plants are usually found sterile, and also because several species are known only from flower or fruit. The key should be used in conjunction with Table 2, where the geographic distribution of all the New World species of the Geminata clade is listed. Where two species differ radically in distribution but key out with the same lead, I have indicated their distribution in parentheses after the species name, e.g., couplet 5.

KEY TO THE NEW WORLD SPECIES OF THE GEMINATA CLADE, INCLUDING THE *SOLANUM HAVANENSE* SPECIES GROUP

1a. Mature leaves glabrous, with no well-developed trichomes more than a single cell long.
  2a. Sympodial units plurifoliate, difoliate, or unifoliate, never geminate.
    3a. Sympodial units plurifoliate or difoliate.
      4a. Inflorescence branched (> 2×).
        5a. Shrubs of river courses; leaves linear to lanceolate . . . . . .
          . . *S. palmillae* Standl. (Mexico); *S. monadelphum* Van Heurk & Müll. Arg. (E slope of Andes)
        5b. Not shrubs of river courses; leaves elliptic to obovate.
          6a. Stems strongly winged . . . . . *S. alatirameum*
          6b. Stems not winged.
            7a. Fruit bright orange, on erect pedicels . . . . . *S. argentinum*
            7b. Fruit green, on deflexed pedicels.
              8a. Sympodial units difoliate or trifoliate.
                9a. Leaves drying black; flowers greenish; plants of SE Brazil . . . *S. cordioides*
                9b. Leaves drying golden abaxially; flowers white; Andes . . . *S. laurifrons*
              8b. Sympodial units plurifoliate.
                10a. Leaves with resinous pit-like domatia in leaf axils abaxially . . *S. pabstii*
                10b. Leaves with no pit-like domatia . . . . . *S. evonymoides*
      4b. Inflorescence simple, or at most furcate.
        11a. Flowers purple.
          12a. Fruit green, conical with a pointed tip; pericarp woody . . . . . *S. conocarpum*
          12b. Fruit blue or blue-green, ellipsoid; pericarp thin.
            13a. New growth completely glabrous . . . . . *S. troyanum*
            13b. New growth pubescent with appressed uniseriate trichomes . . . . . *S. havanense*
        11b. Flowers white.
          14a. Leaves drying golden abaxially.
            15a. Shrubs with pale bark; Cordillera de Avila, Venezuela . . . . . *S. gratum*
            15b. Lax shrubs with reddish brown bark; Andes of Colombia . . . . . *S. humboldtianum*
          14b. Leaves not drying golden abaxially.
            16a. New growth densely arachnoid pubescent, the trichomes soon deciduous; plants of SE Brazil . . . . . *S. canoasense*
            16b. New growth glabrous; plants of N South America and the Caribbean.
              17a. Pedicels strongly winged; Jamaica . . . . . *S. acropterum*
              17b. Pedicels not winged; South America.
                18a. Bark of mature stems pale and shiny; plants of high elevations . . . . . *S. sumacaspi*
                18b. Bark of mature stems reddish brown; plants of low elevations, in river courses . . . . . *S. imberbe* Bitter
    3b. Sympodial units unifoliate.
      19a. Inflorescence axis filiform; fruiting pedicels elongate.
        20a. Stems glabrous.
          21a. Inflorescence axis elongate, to 30 cm; leaf margins ruffled; W Andean slopes . . . . . *S. leptorhachis* Bitter
          21b. Inflorescence congested, < 1 cm; leaf margins plane; Andean Peru . . . . . *S. habrocaulon* S. Knapp
        20b. Stems variously pubescent, the trichomes usually golden.
          22a. Inflorescence axis elongate, to 30 cm; petals planar at anthesis . . . . . *S. leptorhachis*
          22b. Inflorescence axis shorter; petals reflexed at anthesis.
            23a. Inflorescence 1- to 5-flowered.
              24a. Leaf bases acute . . . . . *S. pertenue* Standl. & C. V. Morton
              24b. Leaf bases oblique . . . . . *S. darienense* S. Knapp
            23b. Inflorescence 5- to 15-flowered, more elongate.
              25a. Stem trichomes 1–1.5 mm, usually curling . . . . . *S. capillipes* Britton
              25b. Stem trichomes shorter, erect.
                26a. Calyx lobes minute, < 1 mm; plants of the northern Cordilleras in Venezuela and Colombia . . . . . *S. dissimile* C. V. Morton
                26b. Calyx lobes deltate, longer; plants of E Andean slopes.
                  27a. Leaf margins ruffled; inflorescence axis not markedly zigzag . . . . . *S. confine* Dunal
                  27b. Leaf margins plane; inflorescence markedly zigzag . . *S. gonyrhachis*
      19b. Inflorescence axis stout; fruiting pedicels not very elongate.
        28a. New growth variously pubescent, sometimes minutely so.
          29a. Trichomes of new growth golden, erect . . . . . *S. longevirgatum* Bitter
          29b. Trichomes of new growth floccose, arachnoid . . . . . *S. bahianum* (SE Brazil); *S. cornifolium* Dunal (N Andes)
        28b. New growth completely glabrous.

30a. Leaves markedly 3-veined from the base.
31a. Leaf venation with parallel secondary veins, looking like Melastomataceae . . . . . *S. triplinervium* C. V. Morton
31b. Leaf venation not like Melastomataceae . . . . . *S. cyclophyllum* S. Knapp
30b. Leaves not 3-veined from the base.
32a. Leaf margins ruffled; all orders of venation prominent . . *S. ombrophilum* S. Knapp
32b. Leaf margins plane; venation finely parallel or obscure.
33a. Venation obscure; fruiting pedicels erect . . . . . *S. bellum* S. Knapp
33b. Venation finely parallel; fruiting pedicels deflexed . . . . . *S. elvasioides*

2b. Sympodial units difoliate, geminate.
34a. Leaves of a geminate pair not markedly anisophyllous; minor leaves not differing in shape.
35a. Inflorescence axis variously pubescent, the trichomes uniseriate.
36a. Trichomes of inflorescence axis and new growth floccose, arachnoid . . . . . *S. nigricans* (Mexico to Honduras); *S. maturecalvans* Bitter (Andes)
36b. Trichomes of inflorescence axis and new growth erect, usually golden.
37a. Fruit pubescent; leaves always petiolate . . . . *S. oppositifolium* Ruiz & Pav. (Amazonia); . . . . . *S. turgidum* S. Knapp (Peninsula de Paria, Venezuela)
37b. Fruit glabrous; leaves petiolate or more usually sessile . . . . . *S. sessile* Ruiz & Pav.
35b. Inflorescence axis glabrous or papillate.
38a. Leaves usually < 9 cm long, never repand.
39a. Leaves drying golden abaxially.
40a. Bark red and exfoliating; Mexico to Panama . . . . . *S. tuerckheimii* Greenm.
40b. Bark not red and exfoliating; South America . . . . *S. gratum* (Cordillera de Avila, Venezuela); *S. plowmanii* (Ecuador and Peru)
39b. Leaves not drying golden abaxially.
41a. Fruiting pedicels not erect, slightly or strongly deflexed; South America . . . . . *S. stipulatum* Vell. (SE Brazil); *S. barbulatum* (Andes)
41b. Fruiting pedicels erect; Central America.
42a. Calyx lobes long-triangular . . . . . *S. ramonense* C. V. Morton & Standl.
42b. Calyx lobes quadrate to deltate . . . . . *S. roblense* Bitter
38b. Leaves usually > 10 cm long, to 35 cm, often repand.
43a. Inflorescence branched (> 2×).
44a. Stems strongly winged; plants of SE Brazil . . . . . *S. alatirameum*
44b. Stems terete; plants of N South America . . . . . *S. obovalifolium* Pittier ex Benítez . . . (Cordillera de la Costa, Venezuela); *S. robustifrons* (E Andean slope, Amazonia)
43b. Inflorescence simple, or at most furcate.
45a. Calyx lobes petaloid or long-triangular.
46a. Leaves drying pale green; plants of SE Brazil . . . . . *S. warmingii* Hieron.
46b. Leaves drying brownish; plants of Bolivia to Argentina . . . . . *S. heleonastes* S. Knapp
45b. Calyx lobes deltate.
47a. Inflorescence axis glabrous (in *S. lindenii* Rusby resinous); fruit < 2 cm diam. . . . . *S. lindenii* (Peru and Bolivia); . . . . . *S. narcoticosmum* Bitter (Mexico and Central America)
47b. Inflorescence axis papillate; fruit > 2 cm diam. when mature.
48a. Stems winged; buds enclosed in calyx until just before anthesis . . . . . *S. abitaguense* S. Knapp
48b. Stems terete; buds early exserted from calyx . . *S. cucullatum* S. Knapp

34b. Leaves of a geminate pair markedly anisophyllous; minor leaves differing in shape and size.
49a. Fruiting pedicels deflexed at maturity, often from weight of fruit.
50a. Fruiting pedicels stouter, not markedly longer than in flower; inflorescence axis congested, stout.
51a. Flowers 4-merous; anthers loosely connivent, sagittate . . . . . *S. sagittantherum*
51b. Flowers 5-merous; anthers tightly connivent, not sagittate . . . . . *S. leucocarpon*
50b. Fruiting pedicels elongate (longer than in flower); inflorescence axis usually filiform or thin.
52a. Minor leaves stipule-like, very small and orbicular; bark reddish . . . . . *S. leptopodum* Van Heurck & Müll. Arg.
52b. Minor leaves various, not stipule-like; bark pale.
53a. Stems strongly winged; bark white and exfoliating; plants of SE Brazil . . *S. stipulatum*
53b. Stems terete; bark pale, but not exfoliating; plants of montane regions, Central and N South America . . . . . *S. pastillum* S. Knapp (Central America); *S. hypocalycosarcum* Bitter (SW Ecuador)
49b. Fruiting pedicels erect at maturity.
54a. Fruit red or orange (occasionally bright yellow) at maturity; pericarp thin; seeds flattened, pale yellow.
55a. Fruit > 1 cm diam., red or orange-red; flowers > 1 cm diam., the corolla lobes planar . . . . . *S. pseudocapsicum*

55b. Fruit < 1 cm diam., pale orange; flowers < 1 cm diam., the corolla lobes reflexed . . . . . *S. diphyllum*

54b. Fruit green; seeds ovoid, variously brown.

56a. Inflorescence branched.

57a. Inflorescence elongate, the pedicel scars relatively widely spaced; petals strongly reflexed . . . . . *S. sieberi* Van Heurck & Müll. Arg.

57b. Inflorescence congested, the pedicel scars overlapping; petals planar or slightly reflexed.

58a. Fruit glabrous; leaves sessile . . . . . *S. sessile*

58b. Fruit pubescent; leaves petiolate . . . . . . *S. oppositifolium* (Amazonia); *S. turgidum* (Peninsula de Paria, Venezuela)

56b. Inflorescence simple or the flowers sessile.

59a. Calyx lobes long-triangular.

60a. Flowers sessile . . . . . *S. tanysepalum* S. Knapp

60b. Flowers not sessile, an inflorescence axis present.

61a. Inflorescence axis elongate, the pedicel scars spaced; buds ellipsoid . . . . . *S. irregulare* C. V. Morton

61b. Inflorescence axis stout, congested, the pedicel scars overlapping; buds globose.

62a. Rhizomatous shrubs; new growth sometimes golden puberulent; leaves sessile . . . . . *S. arboreum*

62b. Erect shrubs; new growth various; leaves petiolate . . . . . *S. ramonense* (Central America); *S. anisophyllum* Van Heurck & . . . . . Müll. Arg. (E slopes of Andes, Amazonia)

59b. Calyx lobes deltate or rounded.

63a. Leaves with a well-differentiated petiole . . . . . *S. ripense* Dunal

63b. Leaves attenuate at base, the petiole not well-differentiated.

64a. Leaves shiny adaxially, the midrib keeled . . . . . *S. lucens* S. Knapp

64b. Leaves dull adaxially, the midrib not markedly keeled.

65a. Inflorescence axis with tiny golden erect trichomes . . . . . *S. oppositifolium*

65b. Inflorescence axis glabrous or pubescent, the trichomes not golden and erect.

66a. Small trees; buds elliptic . . . . . *S. sieberi*

66b. Rhizomatous shrublets; buds globose . . . . . *S. arboreum*

1b. Mature leaves with at least some well-developed trichomes more than a single cell long.

67a. Trichomes variously branched.

68a. Trichomes of new growth and leaves floccose, arachnoid, the structure and branching not easy to disentangle.

69a. Sympodial units not geminate.

70a. Pubescence dense on leaf undersurface, lamina not visible . . . . . *S. ochrophyllum* Van Heurck & Müll. Arg.

70b. Lamina clearly visible through pubescence of leaf undersurface.

71a. Trichomes in tufts in vein axils abaxially . . . . . *S. laevigatum* Dunal

71b. Trichomes on lamina or veins, not in tufts.

72a. Calyx lobes deltate; fruit sometimes pubescent; N South America . . *S. cornifolium*

72b. Calyx lobes absent, a mere rim; fruit glabrous; S Bolivia, Argentina . . . . . *S. platycypellon* S. Knapp

69b. Sympodial units difoliate, geminate.

73a. Pubescence dense on leaf undersurface, lamina not visible . . . . . *S. ochrophyllum*

73b. Lamina clearly visible through pubescence of leaf undersurface.

74a. Trichomes in tufts in vein axils abaxially, reddish . . . . . *S. laevigatum*

74b. Trichomes scattered on lamina or veins, or if in tufts, the trichomes grayish.

75a. Corolla 1.5–2.2 cm diam.; Andes . . . . . *S. maturecalvans*

75b. Corolla 1–1.3 cm diam.; Mexico to Honduras . . . . . *S. nigricans*

68b. Trichomes of new growth and leaves dendritic or echinoid, with distinct stiff branches.

76a. Inflorescence branched, > 2×, complex.

77a. Fruit orange when ripe, sometimes dirty yellowish orange . . . . . *S. argentinum*

77b. Fruit green or greenish yellow when ripe.

78a. Stems winged.

79a. Leaves geminate, petiolate . . . . . *S. chlamydogynum* Bitter

79b. Leaves not geminate, sessile . . . . . *S. delitescens*

78b. Stems terete.

80a. Sympodial units plurifoliate.

81a. Trichomes loose, weak, with elongate branches.

82a. Pubescence reddish, the branches of the trichomes congested . . . . . *S. venosum* Dunal

82b. Trichomes transparent, the branches of the trichomes loose . . . . . .*S. oblongifolium* Dunal

81b. Trichomes uniseriate, erect, the branches short.

83a. Pubescence gray, the trichomes somewhat floccose; leaf bases truncate and somewhat oblique; Andes . . . . *S. hypaleurotrichum* Bitter

83b. Pubescence golden or reddish; leaf bases acute; SE Brazil, Argentina, and Paraguay . . . . . *S. oblongifolium*

80b. Sympodial units difoliate.

84a. Fruit glabrous . . . . . *S. clivorum* S. Knapp

84b. Fruit pubescent, the trichomes minute and golden.

85a. Corolla 0.8–1 cm diam.; plants dioecious; coastal Ecuador and Peru . . . . . *S. confertiseriatum* Bitter

85b. Corolla 1–1.5 cm diam.; plants not dioecious; Venezuela . . *S. turgidum*

76b. Inflorescence simple or at most furcate.

86a. Fruit red or orange at maturity.

87a. Trichomes mixed simple and dendritic; sympodial units difoliate or trifoliate . . *S. delicatulum*

87b. Trichomes all dendritic; sympodial units difoliate.

88a. Leaves linear . . . . . *S. spissifolium* Sendtn.

88b. Leaves elliptic.

89a. Pubescence dense on both leaf surfaces; trichomes 1–2 mm, reddish brown . . . . . *S. kleinii* L. B. Sm. & Downs

89b. Pubescence denser on undersides; trichomes 0.25–0.5 mm, yellow . . . . . .*S. pseudocapsicum*

86b. Fruit green at maturity.

90a. Leaves sessile or markedly decurrent on the petiole.

91a. Sympodial units not geminate; anthers obovoid; calyx lobes long-triangular . . . . . *S. delitescens*

91b. Sympodial units geminate; anthers ellipsoid; calyx lobes deltate or triangular.

92a. Fruit pubescent; corolla > 1 cm diam.; minor leaves orbicular . . . . . *S. malletii* S. Knapp

92b. Fruit glabrous; corolla < 1 cm diam.; minor leaves elliptic . . . . *S. xanthophaeum* Bitter (Andes); *S. pseudodaphnopsis* (restingas of SE Brazil)

90b. Leaves distinctly petiolate.

93a. Sympodial units unifoliate.

94a. Inflorescence stout, congested; leaves coriaceous, thick . . *S. nutans* Ruiz & Pav.

94b. Inflorescence filiform, the pedicel scars widely spaced; leaves membranous . . . . . *S. naucinum*

93b. Sympodial units difoliate.

95a. Adaxial leaf surface densely pubescent with simple trichomes; leaves large and repand . . . . . *S. superbum* S. Knapp

95b. Adaxial leaf surface variously pubescent or glabrous, the trichomes not simple; leaves not large and repand.

96a. Adaxial leaf surface shiny and glabrous.

97a. Trichomes of the abaxial leaf surface found in tufts in the vein axils.

98a. Seeds flattened, yellowish brown; flowers usually < 1 cm diam. . . . . *S. nudum*

98b. Seeds ovoid, dark brown; flowers > 1 cm diam. . . . *S. smithii*

97b. Trichomes of abaxial leaf surface on lamina and veins, not in tufts.

99a. Trichomes of abaxial leaf surface mostly confined to the veins, echinoid . . . . . *S. microleprodes* Bitter

99b. Trichomes of abaxial leaf surface on the lamina and veins, dendritic.

100a. New growth resinous; Andes . . . . . *S. lindenii*

100b. New growth not resinous; SE Brazil . . . *S. arenarium*

96b. Adaxial leaf surface variously pubescent.

101a. Seeds flattened.

102a. Marginal leaf trichomes with multicellular bases . . *S. cassioides*

102b. No leaf trichomes with multicellular bases.

103a. Leaves bullate . . . . . *S. youngii* S. Knapp

103b. Leaves not bullate.

104a. Leaves densely red papillate and dendritic pubescent, the trichomes weak and transparent . . . . . *S. psychotrioides* Dunal

104b. Leaves with uniseriate trichomes only, not densely papillate . . . . . *S. tovarii* S. Knapp

101b. Seeds ovoid.
105a. Fruit densely pubescent with densely branched trichomes; plants of SE Brazil . . . . . *S. gnaphalocarpon*
105b. Fruit glabrous; plants of the Andes.
106a. Leaf surfaces abaxially yellowish from dense pubescence . . . . . *S. tunariense* Kuntze
106b. Leaf surfaces clearly visible abaxially, the trichomes loose . . . . . *S. callianthum* C. V. Morton

67b. Trichomes simple or at most furcate.
107a. Trichomes of leaf undersurface in tufts in vein axils or tucked along midrib, not on lamina or veins (if so very few trichomes).
108a. Inflorescences branched (> 2×).
109a. Leaves thick and fleshy; plants of high elevations in central Peru . . . . . *S. amblophyllum*
109b. Leaves membranous; plants of lower elevations, coastal regions.
110a. Petals strongly reflexed at anthesis; buds ellipsoid . . . . . *S. sieberi*
110b. Petals campanulate at anthesis; buds globose . . . . . *S. campaniforme* Roem. & Schult.
108b. Inflorescences simple (or at most furcate).
111a. Flowers fleshy, > 1.5 cm diam.; lobes ± planar at anthesis.
112a. Calyx lobes petaloid.
113a. Plants drying black . . . . . *S. caavurana* Vell.
113b. Plants drying pale green . . . . . *S. warmingii*
112b. Calyx lobes deltate, not petaloid.
114a. Fruiting pedicel swollen apically.
115a. Leaf base attenuate . . . . . *S. corumbense* S. Moore
115b. Leaf base acute to cuneate . . . . . *S. leucocarpon*
114b. Fruiting pedicels not markedly swollen apically.
116a. Leaves not geminate . . . . . *S. validinervium* Benítez & S. Knapp
116b. Leaves geminate.
117a. Leaf trichomes reddish, weak and collapsing, on lamina . . . *S. psychotrioides*
117b. Leaf trichomes white, uniseriate and erect . . . *S. oblongum* Ruiz & Pav.
111b. Flowers < 1.5 cm diam.; lobes reflexed at anthesis.
118a. Flower buds ellipsoid (elongate) . . . . . *S. sieberi* (coastal N South America); . . . . . *S. deflexiflorum* Bitter (Andes).
118b. Flower buds globose.
119a. Sympodial units unifoliate . . . . . *S. mapiricum* S. Knapp
119b. Sympodial units difoliate, usually geminate.
120a. Flower buds large; flowers 1.5–2 cm diam.
121a. Trichomes of leaf undersides moniliform; tepuis of Venezuela . . . . . .*S. tepuiense* S. Knapp
121b. Trichomes of leaf undersides uniseriate; Sierra Nevada de Santa Marta . . . . . *S. lasiopodium* Dunal
120b. Flower buds smaller; flowers < 1.5 cm diam.
122a. Flower buds flattened . . . . . *S. barbulatum*
122b. Flower buds globose.
123a. Stems winged . . . . . *S. santosii* S. Knapp
123b. Stems terete.
124a. Bark of twigs white or very pale yellow, even on young stems; veins of leaf undersurface pale.
125a. Stems and inflorescence axis with simple uniseriate trichomes . . . . . *S. gertii* S. Knapp
125b. Stems and inflorescence axis glabrous.
126a. Anthers unequal (3 long and 2 short), the pores never elongating . . . . . *S. pseudoquina*
126b. Anthers all of equal length, the pores lengthening to slits with age.
127a. Inflorescence axis 1.2–6 cm; calyx lobes deltate . . . . . *S. aphyodendron* S. Knapp
127b. Inflorescence axis 3–8 cm; calyx lobes quadrate . . . . . *S. trichoneuron*
124b. Bark dark brown or reddish; veins drying dark.
128a. New growth densely floccose pubescent, the trichomes reddish . . . . . *S. laevigatum*
128b. New growth glabrous or with scattered uniseriate trichomes, not floccose.
129a. Inflorescences of 1 to 2 flowers; calyx lobes long-triangular . . . . . *S. monanthemon*

129b. Inflorescences with > 5 flowers; calyx lobes deltate to triangular.
130a. Inflorescence subumbellate, all flowers in the apical part . . *S. symmetricum* Rusby
130b. Inflorescence with flowers along entire axis . . . . . *S. nudum*
107b. Trichomes of leaf undersurface on veins and lamina, not confined to vein axils.
131a. Pubescence of leaf undersurface on lamina (occasionally also denser on veins).
132a. Some trichomes of leaves and/or new growth with multicellular bases.
133a. Leaves scabrous, like sandpaper to the touch; trichomes unicellular, stiff . . . *S. trachytrichium*
133b. Leaves not scabrous; trichomes multicellular.
134a. Leaves geminate; Andes . . . . . *S. psychotrioides*
134b. Leaves not geminate; Central America and N South America . . . . . *S. vacciniiflorum* Standl. & L. O. Williams
132b. Trichomes uniseriate, without multicellular bases.
135a. Sympodial units unifoliate.
136a. Leaves sessile, decurrent onto the winged stem . . . . . *S. foetens* S. Knapp
136b. Leaves petiolate, the stem tetete . . . . . *S. quebradense* S. Knapp
135b. Sympodial units difoliate, geminate.
137a. Leaves of a geminate pair markedly anisophyllous, differing in size and shape.
138a. Leaf margins plane; trichomes stiff, curled in one direction; fruit yellow . . . . . *S. falconense* S. Knapp
138b. Leaf margins ruffled; trichomes soft, yellowish; fruit green . . . . . *S. erosomarginatum* S. Knapp
137b. Leaves of a geminate pair the same shape, but often differing in size.
139a. Inflorescence branched.
140a. Anthers unequal (3 long and 2 short), the pores never elongating . . . . . *S. reitzii* L. B. Sm. & Downs
140b. Anthers equal, the pores lengthening to slits with age . . . *S. confertiseriatum*
139b. Inflorescence simple or at most furcate.
141a. Anthers unequal (3 long and 2 short), the pores never elongating . . . . . *S. reitzii*
141b. Anthers equal.
142a. Flowers > 1.5 cm diam.
143a. Calyx broadly urceolate, constricted at apex of petiole . . . . . *S. corumbense*
143b. Calyx conical . . . . . *S. tovarii*
142b. Flowers < 1.5 cm diam.
144a. Flower buds ellipsoid . . . . . *S. incomptum* Bitter
144b. Flower buds globose or obovoid.
145a. Flower buds obovoid; plants of the Bolivian yungas . . . . . *S. chalmersii*
145b. Flower buds globose.
146a. Calyx lobes narrowly triangular . . . . . *S. lasiocladum* S. Knapp
146b. Calyx lobes deltate . . . . . *S. malacothrix* S. Knapp (Mexico); *S. intermedium* Sendtn. (Brazil)
131b. Pubescence of leaf undersurface confined to the veins, only occasionally a few trichomes on the lamina.
147a. Sympodial units difoliate to plurifoliate.
148a. Inflorescence branched.
149a. Flowers < 1 cm diam., not fleshy; fruit pubescent with minute golden trichomes . . . *S. confertiseriatum* (W Ecuador and Peru); *S. oppositifolium* (Amazonia)
149b. Flowers > 1 cm diam., fleshy; fruit glabrous.
150a. Leaves sessile . . . . . *S. sessile*
150b. Leaves petiolate . . . . . *S. rovirosanum* Donn. Sm.
148b. Inflorescence simple or at most furcate.
151a. Leaf trichomes reddish, weak and collapsing.
152a. Fruiting pedicels erect; trichomes all uniseriate . . . . . *S. psychotrioides*
152b. Fruiting pedicels deflexed; some trichomes with multicellular bases . . . . . *S. vacciniiflorum*
151b. Leaf trichomes white, uniseriate and erect.
153a. Leaves of a geminate pair anisophyllous, differing in size and shape . . *S. arboreum*
153b. Leaves of a geminate pair not markedly anisophyllous, differing only in size . . . . . *S. dasyneuron* S. Knapp
147b. Sympodial units unifoliate.

154a. Inflorescence axis very elongate and slender, to 30 cm.
155a. Pubescence of leaf undersides minute, the trichomes usually unicellular . . . . . *S. leptorhachis*
155b. Pubescence of leaf undersides of trichomes to 1 mm long, the trichomes uniseriate, multicellular . . . . . *S. nematorhachis* S. Knapp (W Colombia); . . . . . *S. tenuiflagellatum* S. Knapp (Cordillera de la Costa, Venezuela)
154b. Inflorescence not elongate and slender, usually < 2 cm long.
156a. Leaf apex long acuminate, the ultimate tip blunt.
157a. Flowers 0.4–0.5 cm diam., greenish white; leaf margins ruffled . . . . . *S. yanamonense* S. Knapp
157b. Flowers 0.8–1 cm diam., white; leaf margins plane . . . . . *S. dolosum*
156b. Leaf apex acute to somewhat acuminate, the tip acute.
158a. Stem densely pubescent with yellowish trichomes 1–1.5 mm long . . . *S. capillipes* (Trinidad); *S. valerianum* C. V. Morton & Standl. (Central America)
158b. Stem puberulent, the trichomes very short.
159a. Inflorescence 1- to 3-flowered; plants of Central America . . . *S. pertenue*
159b. Inflorescence > 5-flowered; plants of South America.
160a. Fruiting pedicels 1–1.5× their length in flower; calyx lobes < 0.5 mm long; Andes of Venezuela and Colombia . . . *S. dissimile*
160b. Fruiting pedicels 2–3× their length in flower; calyx lobes ca. 1 mm long; E Andean slopes and Amazonia . . . . . *S. confine*

SYNOPTIC OBSERVATIONS ON THE SPECIES OF THE GEMINATA CLADE

This synoptic set of observations is intended to simplify the task of identifying a member of this large and, at first glance, very uniform group. Sterile plants of members of the Geminata clade are often difficult to identify, and even plants with either flowers or fruit can be difficult. I have included many leaf and whole plant characters so that sterile plants in many cases can be identified to a choice of a few taxa. Once this step has been done, the user can read the descriptions and match distributions in this paper and Knapp (2002a) and consider other characters not used in this list to the plant in hand. All descriptions of members of the Geminata clade are also presented on the Solanaceae Source website (<http://www.nhm.ac.uk/solanaceaesource>). In the following list, geographical regions and then character states are followed by a list of species epithets in alphabetical order. Epithets in parentheses indicate that the state is relatively uncommon in that species or occurs in only some populations of that species. A question mark (?) following a species epithet indicates that the character is likely to occur, but has not been absolutely verified. Epithets for species described in this paper are in italics. Species that vary in a particular character, for example in leaf trichome distribution, will be listed for each state present in that species. Not all relevant characters are considered; for example, I have listed fruit color other than green, but not fruits green—common in most species in the group. In this way, diagnostic states can be easily seen. Many species of the group are rather narrow endemics, occurring in only one country or region. Table 2 lists species recorded for each country of the Neotropics; this list must be used with care, as new collecting is constantly revealing range extensions in these rare forest plants.

Plants of Central America and Mexico: aphyodendron, arboreum, darienense, dasyneuron, diphyllum, imberbe, incomptum, leucocarpon, malacothrix, microleprodes, narcoticosmum, nigricans, nudum, palmillae, pastillum, pertenue, pseudocapsicum, ramonense, roblense, rovirosanum, tuerckheimii, vacciniiflorum, valerianum

Plants of southeastern Brazil: alatirameum, arenarium, bahianum, caavurana, campaniforme, *canoasense*, cassioides, *compressum*, *evonymoides*, gertii, gnaphalocarpon, intermedium, kleinii, pabstii, pseudocapsicum, pseudoquina, reitzii, restingae, santosii, spissifolium, stipulatum, trachytrichium, warmingii

Plants of Caribbean islands (including Trinidad and Tobago): *acropterum*, arboreum, capillipes, *conocarpum*, diphyllum, *havanense*, nudum, pseudocapsicum, sieberi, triste, *troyanum*

Shrubs of river courses, rheophytes (usually with very narrow leaves): amnicola, imberbe, monadelphum, palmillae, spissifolium, stipulatum

Plants growing in weedy thickets at roadsides: acuminatum, aphyodendron, caavurana, *chalmersii*, nudum, rovirosanum, sieberi, *sumacaspi*?, trichoneuron

Pagoda-like architecture (Chamberlain's or Prévost's model with tiers of plagiotropic branches): capillipes, confine, cruciferum?, darienense, dissimile, erosomarginatum, leptopodum, leptorhachis, morii, nematorhachis, pertenue, stipulatum, tuerckheimii, valerianum, yanamonense

Stems markedly winged: abitaguense, chlamydogynum, delitescens, foetens, goniocaulon, *humboldtianum*, oblongifolium

Bark of older stems exfoliating: incomptum, *sumacaspi*, tenuiflagellatum, tuerckheimii, vacciniiflorum

Sympodial units unifoliate: bahianum, bellum, capillipes, confine, cyclophyllum, darienense, dolosum, *elvasioides*, foetens, gonyrhachis, habrocaulon, *humboldtianum*, leptorhachis, longevirgatum, marantifolium, *naucinum*, nematorhachis, nutans, ochrophyllum, ombrophilum, pertenue, stipulatum, triplinervium, unifoliatum, valerianum, yanamonense

Plants completely glabrous, any trichomes not visible to the naked eye: *acropterum*, alatirameum, cordioides, diphyllum, *elvasioides*, evonymoides, pseudocapsicum (cultivated), *sumacaspi*, *troyanum*, warmingii

New growth resinous: amblophyllum, lindenii

Trichomes of new growth reddish when dry: (abitaguense), anisophyllum, arboreum, bahianum, (cucullatum), laevigatum, (nutans), psychotrioides, robustifrons, *sagittantherum*, superbum, vacciniiflorum, venosum

Leaf margins markedly undulate (ruffled): capillipes, confine, cruciferum, darienense, dissimile, erosomarginatum, leptopodum, leptorhachis, morii, nematorhachis, ombrophilum, pastillum, pertenue, stipulatum, valerianum, yanamonense

Leaf pubescence felty (arachnoid trichomes): bahianum, *canoasense*, cornifolium, hypaleurotrichum, laevigatum, maturecalvans, nigricans, ochrophyllum, platycypellon

Leaves bullate or markedly rugose: amblophyllum, maturecalvans, vacciniiflorum, youngii

Leaves drying black: alatirameum, caavurana, campaniforme, cordioides, dasyneuron, foetens, (lasiopodium), narcoticosmum, (symmetricum), (trachytrichium), *troyanum*

Leaves 3-veined from the base: cyclophyllum, triplinervium

Leaves with strongly parallel venation: *elvasioides*, triplinervium

Leaves sessile: alatirameum, delitescens, malletii, sessile, xanthophaeum

Leaves of a geminate pair anisophyllous, markedly unequal in size and differing in shape: anisophyllum, arboreum, erosomarginatum, hypocalycosarcum, irregulare, leptopodum, leucocarpon, lucens, malletii, ombrophilum, *sagittantherum*, tuerckheimii

Leaf and stem trichomes echinoid or Christmas-tree-like: *compressum*, gnaphalocarpon, hypaleurotrichum, nutans, *pseudodaphnopsis*, xanthophaeum

Trichomes dendritic or antler-like: arenarium, argentinum, callianthum, chlamydogynum, clivorum, *compressum*, confertiseriatum, *delicatulum*, erosomarginatum, gnaphalocarpon, kleinii, leucocarpon, lindenii, naucinum, (nigricans), nutans, oblongifolium, oblongum, pseudocapsicum, *pseudodaphnopsis*, smithii, spissifolium, superbum, triste, tunariense, youngii

Leaf trichomes simple, long and uniseriate: capillipes, *chalmersii*, (confertiseriatum), confine, corumbense, dasyneuron, erosomarginatum, falconense, foetens, incomptum, irregulare, lasiocladum, longevirgatum, malacothrix, monanthemon, nematorhachis, quebradense, superbum, tepuiense, tovarii, turgidum, valerianum

Leaf trichomes with enlarged bases: cassioides, trachytrichium, (psychotrioides), vacciniiflorum

Tufts of trichomes in the vein axils abaxially: acuminatum, aphyodendron, barbulatum, caavurana, campaniforme, *compressum*, cornifolium, laevigatum, lasiocladum, lasiopodium, leucocarpon, *monanthemon*, nudum, pseudoquina, psychotrioides, santosii, smithii, symmetricum, tepuiense, trichoneuron

Pit-like domatia in vein axils abaxially: *pabstii*

Pubescence uniform and dense on leaf undersides: arenarium, *argentinum*, callianthum, chlamydogynum, (*compressum*), (confertiseriatum), (corumbense), delitescens, erosomarginatum, gnaphalocarpon, hypaleurotrichum, (leucocarpon), longevirgatum, malacothrix, malletii, microleprodes, nutans, (oblongifolium), oblongum, ochrophyllum, pseudocapsicum, reitzii, superbum, tovarii, tunariense, valerianum, venosum, xanthophaeum, youngii

Leaves shiny adaxially, uniformly pubescent on the lamina abaxially: arenarium, lindenii, *pseudodaphnopsis*, tunariense

Leaves golden abaxially when dry (herbarium specimens): goniocaulon, gratum, *humboldtianum*, laurifrons, (plowmanii), tuerckheimii

Inflorescence branched many times: (acuminatum), alatirameum, amblophyllum, chlamydogynum, clivorum, *compressum*, confertiseriatum, cordioides, delitescens, *evonymoides*, hypaleurotrichum, (lasiopodium), laurifrons, monadelphum, (malletii), oblongifolium, (ombrophilum), oppositifolium, pabstii, (reitzii), (robustifrons), rovirosanum, sessile, (smithii), (tenuiflagellatum), triste, (turgidum), venosum, youngii

Flowers sessile, no inflorescence axis present (if so, very small): *canoasense*, cruciferum, delicatulum, dolosum, elvasioides, monanthemon, pertenue, tanysepalum

Inflorescence filiform, longer than 4 cm: gonyrhachis, leptorhachis, (*naucinum*), nematorhachis, stipulatum, tenuiflagellatum

Corolla green: cordioides, malletii, xanthophaeum, yanamonense

Corolla purple: *conocarpum*, *havanense*, *troyanum*

Corolla campanulate: campaniforme, *canoasense*, cassioides, (trachytrichium), validinervium

Corolla lobes strongly reflexed at anthesis: bellum, capillipes, *chalmersii*, cruciferum, deflexiflorum, diphyllum, dolosum, erosomarginatum, lasiocladum?,

leptopodum, morii, ombrophilum, pertenue, sieberi, valerianum, yanamonense

Corolla lobes tomentose (not merely papillose on the tips and margins) without: arenarium, callianthum, chlamydogynum, clivorum, *compressum*, cornifolium, delitescens, gnaphalocarpon, hypaleurotrichum, kleinii, (lasiocladum), malletii, microleprodes, nutans, (quebradense), superbum, (triste), venosum, (xanthophaeum)

Calyx lobes petaloid or long-triangular: *acropterum*, caavurana, (arboreum), *delicatulum*, irregulare, marantifolium, pseudocapsicum, ramonense, spissifolium, warmingii

Calyx lobes orbicular (live plants): pastillum, *sumacaspi*

Anthers of unequal lengths (2 long, 3 short): pseudoquina, reitzii

Anthers strongly connivent: alatirameum, corumbense, *evonymoides*, leucocarpon, lindenii, (oblongum)

Anthers obovoid in shape, the base narrow: delitescens

Anthers sagittate at the base: amblophyllum, *chalmersii*, (*havanense*), *sagittantherum*

Anther pores small, never elongating to slits with age: *havanense*, pseudoquina, reitzii, *troyanum*

Fruiting pedicels erect: amnicola, anisophyllum, arboreum, bellum, cyclophyllum, diphyllum, kleinii, laevigatum, lucens, marantifolium, pseudocapsicum, ramonense, ripense, roblense, robustifrons, *sagittantherum*, spissifolium, triplinervium, turgidum, unifoliatum

Berries pubescent: abitaguense, callianthum, chlamydogynum, confertiseriatum, cornifolium, cucullatum, gnaphalocarpon, malletii, triste, turgidum

Berries pointed at the tip: *conocarpum*, nigricans, stipulatum

Berries $>$ 3 cm at largest axis: abitaguense, *conocarpum*, cucullatum, superbum

Berries red or orange at maturity: *argentinum*, *delicatulum*, kleinii, pseudocapsicum, spissifolium

Berry purple or bluish: *acropterum*?, *havanense*, *troyanum*

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APPENDIX 1. List of taxa in the Geminata clade treated in this paper. Collections of these 18 species are cited in Appendix 2, while other species in the clade are treated only in Table 1, the key, and the synoptic observations. The numbering system here applies to Appendices 1 and 2 only.

Solanum havanense species group
1. *Solanum acropterum* Griseb.
2. *Solanum conocarpum* Dunal
3. *Solanum havanense* Jacq.
4. *Solanum troyanum* Urb.

Solanum pseudocapsicum species group
5. *Solanum argentinum* Bitter & Lillo
6. *Solanum delicatulum* L. B. Sm. & Downs

Solanum nudum species group
7. *Solanum chalmersii* S. Knapp
8. *Solanum monanthemon* S. Knapp
9. *Solanum pabstii* L. B. Sm. & Downs

Solanum leucocarpon species group
10. *Solanum evonymoides* Sendtn.

Solanum amblophyllum species group
11. *Solanum elvasioides* S. Knapp

Solanum arboreum species group
12. *Solanum humboldtianum* Granados-Tochoy & S. Knapp
13. *Solanum sagittantherum* Granados-Tochoy & C. I. Orozco

Solanum nigricans species group
14. *Solanum canoasense* L. B. Sm. & Downs

Solanum arenarium species group
15. *Solanum compressum* L. B. Sm. & Downs
16. *Solanum pseudodaphnopsis* L. A. Mentz & Stehmann

Solanum dolosum species group
17. *Solanum naucinum* S. Knapp

Species incertae sedis
18. *Solanum sumacaspi* S. Knapp

APPENDIX 2. Exsiccatae for species treated in detail in this paper.

Acevedo, P. R. 5437 (2); *Ackermann, M. & Kollehn, D. 289* (18); *Adams, C. D. 6333* (3), *11833* (3), *12827* (4), *11230* (4); *Aguilar, R. 161* (5); *Aguilar, R. M. 304* (5); *Alain, Bro. 2394* (3), *2396* (3), *4292* (3), *5280* (3); *Almeida, J. 16* (10); *Alvarez, M. R. 226* (5); *Amorim, A. M. 653* (10); *Araque M., J. & Barkley, F. A. 19Ar482* (5); *Asprey, G. F. 2353* (3).

Baer, G. A. 110 (5); *Bailey, L. H. 731* (3); *Bartlett, H. H. 19631* (5), *19750* (5), *19683* (5), *19888* (5), *20321* (5), *20474* (5); *Bastian, E. 1347* (5); *Beck, S. G. 6459* (5), *24480* (7); *Bernardi, L. 20283* (5); *Bianchi, E. M. 12* (5); *Blanchet, J. S. 3096* (10), *3096A* (10); *Borsini, O. 744* (5), *6721* (5); *Bourdy, G. 2017* (5); *Brito, H. S. & Da Vinha, S.G. 117* (10); *Brito, T. H. & Judziewicz, E. J. 3986* (10); *Britton, N. L. 401* (3), *591* (4), *891* (3), *1073* (3), *1511* (3), *2831* (3), *2294* (1), *2294* (1), *2807* (1), *4137* (1), *4213* (3); *Britton, N. L. & Earle, F. S. 7609* (3); *Britton, N. L. & Hollick, A. 2058* (3), *2063* (3), *2807*

(1); *Brizuela, A. 523* (5), *893* (5), *1145* (5), *1334* (5); *Brizuela, J. 166* (5), *744* (5); *Brunt, M. 2200* (3); *Buchtien, O. 7472* (7); *Buratovich, F. 446* (5); *Burkart, A. 2585* (5); *Burkart, A. & Troncoso, N. 12017* (5); *Butzke, A. et al. 7468* (15).

Cabrera, A. L. et al. 31020 (5), *31427* (5); *Cadena, J. J. 78* (12); *Calatayud, G. et al. 966* (18); *Callejas, R. et al. 1618* (10); *Cárdenas, M. 2525* (5), *4754* (5); *Carvalho, A. M. de et al. 4121* (10), *6672* (10); *Castellanos, ? 14825* (5); *Charpin, A. 25819* (5), *23928* (5); *Charpin, A. & Lazare, J. J. 23928* (5); *Charpin, A. S. & Novara, L. 20717* (5); *Claussen, P. s.n.* (10), *200* (10); *Combs, R. 114* (3); *Comisión para la Medición de un Arco de Meridiano 896* (5); *Cordini, J. I. 67* (5); *Cozzo, ? 77* (5); *Curtiss, A. H. 716* (3); *Custodio Filho, A. 265* (10).

Degen, R. & Mereles, F. 3149 (5), *3176* (5), *3179* (5); *Descole, ? 1494* (5); *Dos Santos, E. B. & Alves, M. C. 224* (10); *Dos Santos, T. S. 1051* (10).

Eiten, G. & Clayton, W. D. 5790 (6); *Ekman, E. L. 10555* (3), *12876* (3); *Euponino, A. & Da Vinha, S. G. 481* (10).

Farfán, J. et al. 373 (18); *Folli, D. A. 1040* (10); *Fries, R. E. s.n.* (5); *Fuentes, A. & Novarro, G. 1958* (5).

Glaziou, A. F. M. 8856 (10); *Gramaja, A. 16* (5); *Granados-Tochoy, J. C. et al. 389* (12), *844* (12), *860* (12), *888* (13).

Harris, W. 8530 (4), *8681* (4), *9000* (4), *10036* (3), *10231* (3), *10237* (3), *10298* (1), *10718* (3), *10752* (1); *Harris, W. & Britton, N. L. 10723* (1); *Hatschbach, G. 382* (16), *10198* (16), *15030* (9), *22679* (15), *22707* (9), *30713* (15); *Hatschbach, G. & Pereira, E. 13071* (15); *Hatschbach, G. et al. 72547* (9); *Hawkes, J. G. 36* (12); *Hayward, K. J. 2078* (5); *Herb. Bot. Dept. Jamaica 6175* (3), *6248* (3); *Herb. Hoehne 38942* (5); *Hoehne, F. C. 3042* (10); *Hoehne, W. s.n.* (6), *s.n.* (6); *Howard, R. A. 5740* (3), *6590* (3), *15024* (1); *Howard, R. A. & Proctor, G. R. 13765* (3), *14365* (4); *Huidobro, A. M. R. 617* (5), *536* (5), *3045* (5); *Humboldt, F. W. H. A. & Bonpland, A. s.n.* (12); *Hunziker, A. T. 1064da* (5).

Ibarrola, T. S. 3854 (6); *Ibisch & Ibisch 93.1477* (8); *Idrobo, J. M. 5437* (12); *Itaipú Binacional 668* (5).

Jack, J. G. 7317 (3); *Jardim, A. & Rosas-Hurtado, N. 1550* (5); *Jiménez, J. J. 1907* (3); *Jorgensen, P. 2622* (5); *Jørgensen, P. M. et al. 44* (11).

Kallunki, J. A. et al. 706A (10); *Kegler, A. 286* (15), *1301* (15), *1325* (15); *Killeen, T. et al. 4209* (5); *Klein, R. 4567* (9); *Krapovickas, A. & Cristóbal, C. 17526* (5), *27143* (5), *27157* (5), *30423* (5), *46303* (5); *Krapovickas, A. & Schinini, A. 30909* (5), *31246* (5), *39269* (5); *Krapovickas, A. et al. 28017* (5), *28397* (5), *56552* (5); *Kummrow, R. 2410* (14); *Kuroiwa, N. & Maeda, A. 2332* (5).

León, Bro. 7135 (3); *Lewis, C. B. s.n.* (1); *Lillo, M. 3343* (5), *9837* (5); *Lima, J. C. A. & Messias Santos, M. 144* (10); *Lindeman, J. C. & Haas, J. H. de 4915* (15), *5302* (15); *Lozano-C., G. 98* (12); *Luna, F. E. 1049* (5); *Luno, M. Y. de 24* (5); *Luteyn, J. L. & Dorr, L. J. 13722* (7).

MacFadyen, J. s.n. (3); *Madsen, J. E. et al.* (11); *Maldonado, R. 227* (5); *Malvárez, M. R. 305* (5), *497* (5), *653* (5), *691* (5); *Malvárez, R. 155* (5), *1335* (5), *1401* (5); *March, W. T. 1666* (3); *Martins, F. R. et al. 12361* (10); *Martius, C. F. P. von 625* (10); *Mattos Silva, L. A. et al. 376* (10), *1298* (10), *1378* (10); *Maxon, W. R. 2206* (4); *Mentz, L. A. & Sobral, M. 188* (9), *215* (15); *Mèrat de Vaumartoise, F. V. s.n.* (3); *Mereles, F. & Degen, R. 5570* (5), *5616* (5), *5642* (5), *5859* (5), *6114* (5); *Mexia, Y. 4341* (5), *7834* (5); *Meyer, T. 3652* (5), *3663* (5), *5048* (5), *13127* (5), *9900* (5); *Michel, R. de 2* (5); *Miller, G. S. 1358* (3), *1490* (1); *Morales, G. 497* (12); *Morel, I. 7268* (5), *7350* (5); *Mori, S. A. 12747* (10); *Mori, S. A. et al. 10737* (10), *10813* (10); *Morrone, O. et al. 1388* (15); *Morton, C. V. s.n.* (5); *Murguia R., O. 379* (5), *536* (5).

Nee, M. 35288 (5), *35317* (5), *35330* (5), *40420* (5), *44597* (5), *49083* (5), *51723* (5), *51723* (5), *51149* (5), *51171* (5), *51132* (5), *51320* (5), *51132* (5), *51320* (5), *51337* (5); *Nee, M. & Bohs, L. 50828* (5); *Nee, M. et al. 50736* (5), *51722* (5), *51773* (7), *51777* (7), *51795* (7), *52101* (5); *Norrbom, A. L. 91/A3* (5); *Novara, L. J. 527* (5), *535* (5), *4572* (5), *4856* (5), *5625* (5), *5892* (5), *7248* (5), *7755* (5).

O'Donell, C. A. 3135 (5), *4321* (5), *4357* (5); *Orozco, C. I. 1062* (12); *Ousset, ? 112* (5).

Pabst, G. & Pereira, E. 6123 (9), *6200* (9), *6281* (9), *6693* (15); *Partridge, W. 61701* (6); *Pedersen, T. M. 2585* (5); *Penseiro, J. & Mariano, D. 4323* (5); *Pérez, L. 173* (15); *Perkins, J. R. 1375* (4); *Pierotti, S. A. s.n.* (5), *2* (5), *4012* (5); *Pinheiro, R. S. 1490* (10); *Pirani, J. R. et al. 3028-A* (10); *Plowman, T. C. 2729* (5), *3241* (3), *2986* (3), *3241* (3), *3256* (4); *Poeppig, E. F. s.n.* (3); *Proctor, G. R. 6104* (3), *7980* (4), *9911* (4), *16625* (3), *20480* (4), *21339* (4), *23668* (3), *24845* (4), *26410* (4), *31565* (3), *31768* (4), *33808* (4); *Proctor, G. R. & Stearn, W. T. 11802* (1); *Purdie, W. s.n.* (1), *135* (3).

Quintero, J. 63 (5).

Ragonese, ? 274 (5), *3000* (5); *Rambo, B. 44621* (15), *52193* (15); *Read, R. W. 1918* (4); *Reitz, P. R. & Klein, R. 7867* (15), *11438* (6), *14584* (15), *15947* (15); *Ribas, O. S. & Silva, J. M. 190* (9); *Richard, L. C. s.n.* (2); *Risso, J. L. 156* (5), *295* (5); *Risso, L. 239* (5); *Rodriguez, F. 21* (5); *Rodriguez, F. M. 249* (5); *Rojas, T. 14486a* (5); *Rossato, M. et al. 2484* (15); *Rugel, F. 94* (3), *144* (3); *Ruthsatz, B. 364/2* (5).

Sagra, R. de la s.n. (3), *294* (3); *Sangster, I. s.n.* (3); *Santos, F. C. 73* (10); *Schafer, J. A. 13795* (3); *Schulz, A. G. 2041* (5), *3839* (5), *8170* (5), *15635* (5), *15825* (5), *17038* (5); *Sehnem, A. 2203* (9), *3934* (9), *12505* (15); *Seidel, R. & Richtre, E. 851* (7); *Sellow, F. s.n.* (10), *244* (10), *819* (10); *Sessé, M. 219* (3), *535* (3); *Siñani 261* (7); *Smith, D. N. & Canne, J. 5793* (17); *Smith, L. B. & Klein, R. M. 13489* (9); *Sobral, M. et al. ICN 7991* (15), *111138* (9); *Solomon, J. C. 10579* (5); *Soria, N. 364* (5), *394* (5); *Spichinger, R. RS2185* (5); *Stearn, W. T. 140* (3), *319* (3), *499* (1), *537* (3), *634* (3), *865* (3), *821* (3), *922* (4); *Stehmann, J. R. 728* (15), *1524* (15), *1549* (9), *1756* (14); *Stehmann, J. R. & Sobral, M. ICN 111362* (15), *ICN 111359* (15); *Stuckert, T. 8170* (5), *8896* (5), *15164* (5), *16820* (5), *17237* (5), *18156* (5), *19721* (5).

Thomas, W. W. et al. 9926 (10), *11202* (10); *Tressens, S. G. et al. 5910* (15).

Valenzuela, L. et al. (18); *Vanni, R. et al. 2362* (5), *4334* (5); *Varela, F. 1545* (5); *Vargas C., I. G. & Tapia, E. 1052* (5); *Vargas C., I. G. et al. 3138* (8); *Venturi, S. 2434* (5), *2655* (5), *5797* (5), *7774* (5), *7857* (5), *9818* (5); *Villafañe, T. 74* (5).

Wasum, R. 270 (9), *1174* (15), *1422* (15), *9015* (15); *Waters, J. s.n.* (1); *Webster, G. L. 3700* (3), *13669* (4); *Webster, G. L. & Proctor, G. R. 5546* (1); *Webster, G. L. et al. 51* (3), *8076* (3); *Weisz, P. BAA 5761* (5); *Whitefoord, C. 1399* (4); *Wilson, N. s.n* (3); *Wilson, P. 11305* (3); *Wood, J. R. I. 9529* (5), *9955* (7); *Wright, C. 3025* (3); *Wright, W. s.n.* (3).

Yuncker, G. 18423 (3).

APPENDIX 3. Specimens used in the analysis of pubescence in *Solanum argentinum*. Collections are listed by senior collector only (for complete data, see Appendix 2), and duplicates in different herbaria are listed separately. ARG = Argentina; BOL = Bolivia; PGY = Paraguay. Sample sizes for latitude and longitude = 160; elevation = 70. Specimens for which an elevation was not given were not included in the analysis for altitude. Digital latitude and longitudes are rounded to two decimal places.

Collector	Number	Location	Digital latitude	Digital longitude	Elevation (m)	Score
Aguilar, R. M.	304	ARG	−25.17	−64.17		1
Alvarez, M. R.	226	ARG	−25.17	−64.17		1
Baer, G. A.	110	ARG	−26.60	−65.30		1
Bartlett, H. H.	19631	ARG	−25.50	−64.30		1
Bartlett, H. H.	19750	ARG	−28.25	−63.92		1
Bartlett, H. H.	20474	ARG	−24.78	−64.15		1
Bartlett, H. H.	19888	ARG	−18.38	−62.92		2
Bartlett, H. H.	19683	ARG	−19.27	−62.47		3
Bartlett, H. H.	20321	ARG	−29.22	−65.45		3
Bastian, E.	1347	BOL	−29.12	−65.35	1800	3
Beck, S. G.	6459	BOL	−28.62	−65.80		1
Bernardi, L.	20283	PGY	−24.82	−65.45		2
Bernardi, L.	20283	PGY	−20.05	−63.53		2
Bourdy, G.	2017	BOL	−26.08	−65.97		1
Brizuela, A.	1145	ARG	−26.08	−65.97		1
Brizuela, A.	523	ARG	−23.33	−64.25		1
Brizuela, A.	893	ARG	−22.33	−62.62		1
Brizuela, J.	744	ARG	−22.30	−62.53		1
Buratovich, F.	446	ARG	−22.30	−62.53		2
Burkart, A.	12017	ARG	−22.58	−63.33	1400	3
Cabrera, A. L.	31020	ARG	−22.30	−62.53		3
Cabrera, A. L.	31427	ARG	−26.08	−65.97		3
Cárdenas, M.	4754	BOL	-17.58	−62.67	900	1
Charpin, A.	20717	ARG	−26.10	−64.32	1700	1
Charpin, A.	25819	ARG	−26.10	−64.32		3
Charpin, A.	25819	ARG	26.08	−65.97		3
Charpin, A. S.	20717	ARG	−25.25	−65.42	1700	1
Comisión para la Medición de un Arco de Meridiano	896	ARG	−27.28	−65.00		2
Cordini, J. I.	67	ARG	−27.92	−63.90		2
Cozzo, ?	77	ARG	−27.92	-63.90		1
Degen, R.	3149	PGY	−25.17	−64.17		1
Degen, R.	3149	PGY	−26.82	−65.22		1
Degen, R.	3149	PGY	−26.82	−65.22		1
Degen, R.	3176	PGY	−25.70	−63.07		1
Degen, R.	3179	PGY	−22.33	−62.62		1
Fries, R. E.	s.n.	ARG	−22.30	−62.53		1
Fuentes, A.	1958	BOL	−25.17	−65.17	300	1
Gramaja, A.	16	ARG	−25.17	−65.17		1
Gramaja, A.	16	ARG	−25.17	−65.17		1
Hayward, K. J.	2078	ARG	−25.17	−65.17		1
Huidobro, A. M. R.	536	ARG	−18.83	−65.25	713	3
Hunziker, A. T.	1064	ARG	−20.03	−63.52	1200	1
Jardim, A.	1550	BOL	−18.65	−63.25	1385	3
Killeen, T.	4209	BOL	−29.87	−63.73	700	3
Krapovickas, A.	27143	ARG	−18.67	−63.24		1
Krapovickas, A.	27157	ARG	−18.67	−63.24		1
Krapovickas, A.	27157	ARG	−26.88	−64.73		1
Krapovickas, A.	46303	ARG	−28.17	−65.67		1
Krapovickas, A.	56552	ARG	−25.62	−65.47		2
Krapovickas, A.	17526	ARG	−28.20	−65.13		3

APPENDIX 3. Continued.

Collector	Number	Location	Digital latitude	Digital longitude	Elevation (m)	Score
Krapovickas, A.	28017	ARG	−28.17	−65.67		3
Krapovickas, A.	28397	ARG	−28.17	−65.67		3
Krapovickas, A.	30423	ARG	−24.58	−65.58		3
Krapovickas, A.	30909	ARG	−31.40	−64.18	350	3
Krapovickas, A.	30909	ARG	−24.82	−65.45	350	3
Krapovickas, A.	31246	BOL	−32.42	−63.25		3
Krapovickas, A.	39269	ARG	−19.58	−62.58		3
Kuroiwa, N.	2332	BOL	−26.78	−65.25		3
Lillo, M.	3343	ARG	−24.28	−65.10		1
Lillo, M.	3343	ARG	−24.28	−65.10		1
Lillo, M.	9837	ARG	−24.28	−65.10		3
Malvárez, M. R.	653	ARG	−24.28	−65.10		1
Malvárez, R.	1401	ARG	−26.15	−65.30		3
Malvárez, R.	155	ARG	−26.50	−64.92		3
Mereles, F.	5570	PGY	−26.50	−64.92		1
Mereles, F.	5570	PGY	−25.60	−65.63		1
Mereles, F.	5642	PGY	−25.60	−65.63		2
Mereles, F.	5859	PGY	−25.60	−65.63		2
Mexia, Y.	4341	ARG	−30.20	−64.48	760	1
Mexia, Y.	7834	ARG	−34.58	−58.48	762	1
Mexia, Y.	7834	ARG	−21.68	−64.83	762	1
Mexia, Y.	7834	ARG	−29.83	−63.50	762	1
Meyer, T.	3652	ARG	−22.63	−59.67	1260	3
Meyer, T.	5048	ARG	−22.63	−59.67	360	3
Meyer, T.	9900	ARG	−26.90	−60.13		3
Meyer, T.	9900	ARG	−23.22	−64.10		3
Meyer, T.	9900	ARG	−25.20	−58.00		3
Morel, I.	7268	ARG	−24.68	−64.25		3
Murgia, O.	379	BOL	−22.97	−61.83	3400	1
Nee, M.	35288	BOL	−23.28	−61.47	820	1
Nee, M.	49083	BOL	−17.77	−63.18	550	1
Nee, M.	50736	ARG	−17.77	−63.18	425	1
Nee, M.	51723	BOL	−24.07	−64.27	550	1
Nee, M.	51723	BOL	−24.07	−64.27	550	1
Nee, M.	40420	BOL	−25.53	−65.57	420	2
Nee, M.	40420	BOL	−25.53	−65.57	420	2
Nee, M.	35317	BOL	−25.60	−65.30	800	3
Nee, M.	35317	BOL	−24.33	−59.50	800	3
Nee, M.	35330	BOL	−24.33	−59.50	810	3
Nee, M.	44597	BOL	−24.33	−59.50	500	3
Nee, M.	44597	BOL	−27.33	−58.93	500	3
Nee, M.	44597	BOL	−23.43	−60.15	500	3
Nee, M.	44597	BOL	−26.48	−65.37	500	3
Nee, M.	50828	ARG	−25.25	−65.42	1300	3
Nee, M.	51132	BOL	−24.25	−64.83	810	3
Nee, M.	51132	BOL	−21.67	−64.83	810	3
Nee, M.	51149	BOL	−55.33	−65.17	850	3
Nee, M.	51171	BOL	−24.30	−64.97	920	3
Nee, M.	51320	BOL	−25.50	−68.50	900	3
Nee, M.	51320	BOL	−26.20	−64.78	900	3
Nee, M.	51337	BOL	−26.20	−64.78	850	3
Nee, M.	51722	BOL	−25.30	−64.90	520	3
Nee, M.	51722	BOL	−20.35	−63.47	520	3
Nee, M.	51722	BOL	−20.30	−63.27	520	3
Nee, M.	51722	BOL	−25.38	−65.10		3
Novara, L. J.	527	ARG	−26.47	−65.18		2

Appendix 3. Continued.

Collector	Number	Location	Digital latitude	Digital longitude	Elevation (m)	Score
Novara, L. J.	527	ARG	−24.90	−65.48		2
Novara, L. J.	5625	ARG	−24.75	−65.25		2
Novara, L. J.	5625	ARG	−22.90	−63.98		2
Novara, L. J.	7248	ARG	−22.90	−63.98	1400	2
Novara, L. J.	4856	ARG	−20.75	−63.15	1133	3
Novara, L. J.	4856	ARG	−22.23	−63.70		3
Novara, L. J.	535	ARG	−20.07	−63.54	1180	3
Novara, L. J.	5892	ARG	−24.38	−65.10	1200	3
Novara, L. J.	5892	ARG	−27.50	−59.60	1200	3
Novara, L. J.	7755	ARG	−25.98	−64.95	1150	3
O'Donell, C. A.	4321	ARG	−24.90	−65.48		1
O'Donell, C. A.	3135	ARG	−23.33	−64.25		3
O'Donell, C. A.	4357	ARG	−24.78	−64.23		3
Pierotti, S. A.	s.n.	ARG	−24.78	−64.23		1
Pierotti, S. A.	s.n.	ARG	−24.78	−64.23		1
Pierotti, S. A.	4012	ARG	−25.00	−58.67		2
Pierotti, S. A.	4012	ARG	−20.40	−63.28		2
Pierotti, S. A.	4012	ARG	−20.40	−63.28		2
Pierotti, S. A.	2	ARG	−20.43	−63.28		3
Plowman, T. C.	2729	ARG	−18.13	−63.20	77	3
Plowman, T. C.	2729	ARG	−18.13	−63.20	77	3
Quintero, J.	63	ARG	−18.13	−63.20		1
Ragonese, ?	274	ARG	−18.13	−63.20		3
Ragonese, ?	3000	ARG	−23.56	−64.97		3
Risso, J. L.	156	ARG	−20.09	−63.52		1
Risso, J. L.	295	ARG	−20.09	−63.52	900	1
Ruthsatz, B.	364	ARG	−20.05	−63.53	2120	1
Schulz, A. G.	17038	ARG	−20.28	−63.45		2
Schulz, A. G.	15635	ARG	−19.90	−63.53		3
Schulz, A. G.	15825	ARG	−19.90	−63.53		3
Schulz, A. G.	3839	ARG	−19.94	−63.52		3
Schulz, A. G.	8170	ARG	−18.14	−63.20		3
Solomon, J. C.	10579	BOL	−18.14	−63.20		3
Spichinger, R.	2185	PGY	−18.13	−63.19	100	2
Stuckert, T.	15164	ARG	−18.13	−63.19		1
Stuckert, T.	15164	ARG	−25.60	−65.63		1
Stuckert, T.	16820	ARG	−25.60	−65.63		1
Vanni, R.	4334	ARG	−24.78	−64.88		3
Vargas, I. G.	1052	BOL	−25.75	−55.38	400	1
Venturi, S.	2434	ARG	−25.75	−55.38	550	1
Venturi, S.	5797	ARG	−23.93	−62.55	500	1
Venturi, S.	5797	ARG	−23.33	−64.25	500	1
Venturi, S.	5797	ARG	−24.78	−65.02	500	1
Venturi, S.	5797	ARG	−23.33	−64.25	500	1
Venturi, S.	7774	ARG	−27.47	−58.83	1000	1
Venturi, S.	7857	ARG	−27.47	−58.83	800	1
Venturi, S.	7857	ARG	−24.22	−67.07	800	1
Venturi, S.	9818	ARG	−28.38	−62.12	1300	1
Venturi, S.	9818	ARG	−26.83	−61.00	1300	1
Venturi, S.	9818	ARG	−27.33	−58.93	1300	1
Venturi, S.	2655	ARG	−27.33	−58.93	800	2
Villafañe, T.	74	ARG	−27.57	−60.53		1
Weisz, P.	5761	ARG	−21.82	−64.67		1
Wood, J. R. I.	9529	BOL	−24.25	−61.25	1900	1

PHYLOGENETIC RELATIONSHIPS OF TWO ENDEMIC GENERA FROM EAST ASIA: *TRICHOCOLEOPSIS* AND *NEOTRICHOCOLEA* (HEPATICAE)[1]

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Abstract

Trichocoleopsis S. Okamura and *Neotrichocolea* S. Hatt. are two water saccate liverwort genera endemic to East Asia whose systematic positions have been controversial. To address their phylogenetic positions and relationships within liverworts, two data sets were analyzed using maximum parsimony and maximum likelihood methods: (1) four genes (*psbA*, *rps4*, 26S, and *nad5*) of 57 taxa, and (2) nine genes (chloroplast small subunit [SSU], large subunit [LSU], *atpB*, *rbcL*, *psbA*, and *rps4*; nuclear ribosomal 18S and 26S; mitochondrial *nad5*) of 24 taxa. All of our analyses suggested the sister relationship between *Trichocoleopsis* and *Neotrichocolea*, which is also supported by morphological characters, such as a lobed leaf with ciliate margins, water sacs, coelocaules, and interior capsule wall cells with thin walls. Traditional classifications usually considered *Trichocoleopsis* and *Neotrichocolea* as members of Lepidolaenaceae or Trichocoleaceae, whereas our results strongly suggested that the *Trichocoleopsis–Neotrichocolea* clade allied with Ptilidiaceae, which is supported by the similar ciliate and lobed leaves. However, considering many differences between them, such as in the perianth, sporeling type, water sacs, and rhizoids, we support a family rank for the Neotrichocoleaceae, containing *Trichocoleopsis* and *Neotrichocolea*, as previously suggested by Inoue in 1974.

Key words: East Asia, endemic, multiple genes, *Neotrichocolea*, Neotrichocoleaceae, phylogeny, *Trichocoleopsis*.

Trichocoleopsis S. Okamura and *Neotrichocolea* S. Hatt. are two liverwort genera from East Asia that are considered to be related based on the shared characteristic of bearing water sacs on their leaves. *Trichocoleopsis* contains two species, *T. sacculata* (Mitt.) S. Okamura and *T. tsinlingensis* P. C. Chen ex M. X. Zhang, and the latter is only distinguished from the former by its entire or only 1- to 3-ciliate lobe margins (Zhang, 1982). *Neotrichocolea* is a monotypic genus with *N. bisseti* (Mitt.) S. Hatt. The two genera are restricted to moist forests in China, Japan, Korea, Burma, and the Russian Far East. As habitat destruction increases, all three species of both genera are seriously threatened and on the verge of extinction (Wu et al., 1997; Cao et al., 2006). An updated distribution map of these species is shown in Figure 1. This map is based on our own consultations of specimens as well as other published research (see Table 1).

In 1891, Mitten published two species from Japan, *Blepharozia sacculata* Mitt. and *Mastigophora bisseti* Mitt; the former is the basionym of *Trichocoleopsis sacculata* and the latter is the basionym of *Neotrichocolea bisseti*. Thereafter, the two species were transferred to *Ptilidium* Nees (Stephani, 1897; Evans, 1905) before Okamura (1911) and Hattori (1947) established the monotypic genera *Trichocoleopsis* and *Neotrichocolea*, respectively. In addition, Horikawa (1934) suggested that *N. bisseti* be placed in the genus *Trichocoleopsis*.

Since their establishment, the systematic positions of these two genera have been controversial. *Trichocoleopsis* has been placed in Ptilidiaceae (Evans, 1939; Hattori, 1947; Mizutani & Hattori, 1969), Trichocoleaceae (Nakai, 1943; Müller, 1951–1958; Schuster, 1957; Mizutani, 1972), or Lepidolaenaceae (Schuster, 1966); meanwhile, *Neotrichocolea* has been recognized as a member of Ptilidiaceae (Hattori, 1947;

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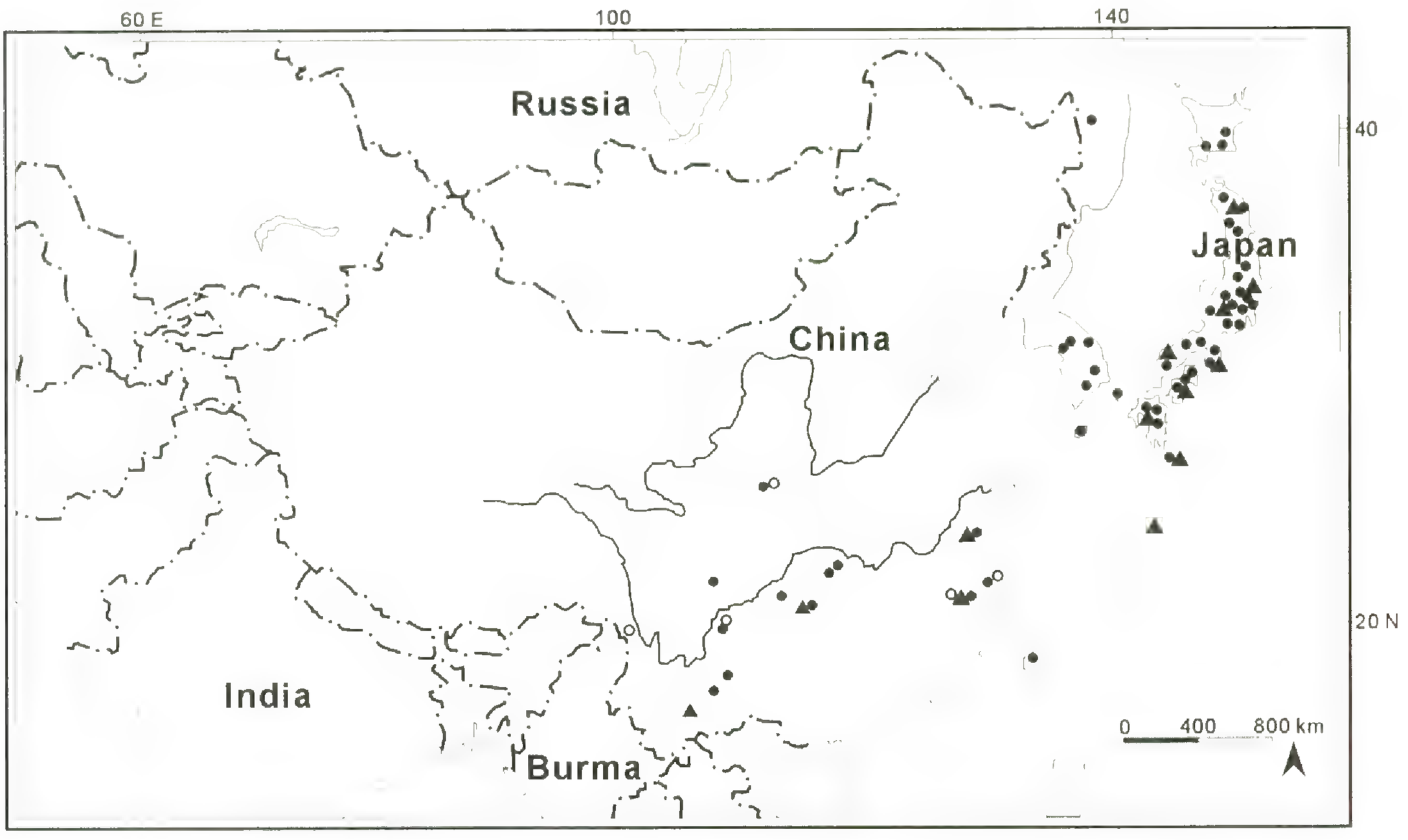

Figure 1. Distribution map of *Trichocoleopsis* and *Neotrichocolea*. Filled circle = *T. sacculata*, open circle = *T. tsinlingensis*, filled triangle = *N. bisseti*.

Table 1. Specimen vouchers for geographic distributions of *Neotrichocolea* and *Trichocoleopsis* in eastern Asia (Fig. 1). Mapped distributions in China and adjacent regions are supported by the specimen vouchers or inferred from the literature (Gao & Cao, 1988, 2000; Hong, 1966 [Korea]; Inoue, 1974 [Japan]; Konstantinova et al., 1992 [Russia]; Koponen et al., 2004; Wu, 1992; Zhang, 1982). Asterisks mark the two specimen vouchers for the new chloroplast and nuclear ribosomal DNA sequences reported in this paper. IFSBH = Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences.

Locality	Collection	Herbarium/ Literature
***Neotrichocolea bisseti* (Mitt.) S. Hatt.**		
Guang mingding, Mt. Huang, Anhui Province, China	*Chen et al. 6900*	PE
Qing liangtai, Mt. Huang, Anhui Province, China	*Chen et al. 7081*	PE
Qing liangtai, Mt. Huang, Anhui Province, China	*Chen et al. 7128*	PE
Shi xinfeng, Mt. Huang, Anhui Province, China	*Chen et al. 7161*	PE
Shi xinfeng, Mt. Huang, Anhui Province, China	*Chen et al. 7104*	PE
Shi xinfeng, Mt. Huang, Anhui Province, China	*Chen et al. 7171*	PE
Shi xinfeng, Mt. Huang, Anhui Province, China	*Chen et al. 7172*	PE
Xi haimen, Mt. Huang, Anhui Province, China	*Chen et al. 7038*	PE
Xi haimen, Mt. Huang, Anhui Province, China (alt. 1600 m)	*Chen et al. 7003*	PE
Lian huafeng, Mt. Huang, Anhui Province, China	*Chen et al. 6939*	PE
Qing liangtai-Er daowan, Mt. Huang, Anhui Province, China	*Chen et al. 7188*	PE
Se-Tse-ling, Mt. Huang, Anhui Province, China (alt. 1800 m)	*Ku, C. M. s.n.*	PE
Bei hai, Mt. Huang, Anhui Province, China (alt. 1600 m)	*Gao, C. H. 21280*	SHM
Bei hai, Mt. Huang, Anhui Province, China (alt. 1600 m)	*Hu, R. L. & Gao, C. H. 8679*	HSNU
Song yanan, Mt. Huang, Anhui Province, China (alt. 1300 m)	*Hu, R. L. & Gao, C. H. 8737*	HSNU
Xi haimen, Mt. Huang, Anhui Province, China	*Chen et al. 9371*	HSNU
Huang gang, Mt. Wuyi, Fujian Province, China	*Gao, C. H. 25077*	SHM
Yuan jiang, Yunnan Province, China		Gao & Cao, 2000
*Mt. Fanjing, Guizhou Province, China	*He, L. 040522*	PE
***Trichocoleopsis sacculata* (Mitt.) S. Okamura**		
Shi zilin, Mt. Huang, Anhui Province, China (alt. 1800 m)	*Chen et al. 6399*	PE
Shi zilin, Mt. Huang, Anhui Province, China	*Chen et al. 6384*	PE

Table 1. Continued.

Locality	Collection	Herbarium/ Literature
Shi zilin-Xihai, Mt. Huang, Anhui Province, China	*Chen et al. 6497*	PE
Xi haimen, Mt. Huang, Anhui Province, China	*Chen et al. 6997*	PE
Qing liangtai, Mt. Huang, Anhui Province, China (alt. 1500 m)	*Chen et al. 7172*	PE
Qing liangtai, Mt. Huang, Anhui Province, China	*Chen et al. 7179*	PE
Shi zilin-Yun gusi, Mt. Huang, Anhui Province, China	*Chen et al. 7428*	PE
Feng huangsi, Mt. Jinfo, Sichuan Province, China	*Chen et al. 1632*	PE
San guandian, Mt. Taibai, Shanxi Province, China (alt. 3600 m)	*Li X. J. 705*	PE
Ba xiantai, Mt. Taibai, Shanxi Province, China (alt. 4000 m)	*Li X. J. 718*	PE
*Qing liangtai, Mt. Huang, Anhui Province, China	*Liu Y. 4170*	PE
Qing liangtai, Mt. Huang, Anhui Province, China	*Li D. K. 02274*	SHM
Qing liangtai, Mt. Huang, Anhui Province, China	*Zheng W. F. 14307*	HSNU
Qing liangtai, Mt. Huang, Anhui Province, China (alt. 1600 m)	*Hu, R. L. & Gao, C. H. 8632*	HSNU
Mt. E mei, Sichuan Province, China	*Gao Q. 19374, 19485*	IFSBH
Mt. E mei, Sichuan Province, China	*Gao C. H. 15808, 15946*	Gao & Cao, 1988
Yang yuping, Mt. Jinfo, Chongqing, China (alt. 1400 m)	*Cao T. & Li Q. 41495*	IFSBH
Po niangyan, Daguan, Yunnan Province, China	*Li X. J. 4320*	KUN
Yi liang, Yunnan Province, China	–	Gao & Cao, 2000
Qu jing, Yunnan Province, China	–	Gao & Cao, 2000
Mt. Wuyi, Fujian Province, China	*Gao C. H. 25099*	Gao & Cao, 1988
Bai shanzu Natural Reserve, Zhejiang Province, China (alt. 1010–1600 m)	*Zhu R. L. 90402, 90413, 90554, 90564, 90839, 90883*	HSNU
Mt. Jiu long, Zhejiang Province, China (alt. 1300 m)	*Liu Z. L. 3686*	HSNU
Mt. Xitianmu, Zhejiang Province, China (alt. 1300 m)	*Wang Y. F. 00405*	HSNU
Mt. Fanjing, Guizhou Province, China	*He L. s.n.*	GACP
Mt. Ba dagong, Hunan Province, China	*Koponen 48115, 48168*	Koponen et al., 2004
Mt. Huping, Hunan Province, China	*Koponen 52188*	Koponen et al., 2004
Tai dong, Taiwan Province, China	–	Wu, 1992
***Trichocoleopsis tsinlingensis* P. C. Chen ex M. X. Zhang**		
Mt. Taibai, Shanxi Province, China	*Wei Z. P. 5538*	KUN
Fang yangsi-Ping ansi, Mt. Taibai, Shanxi Province, China (alt. 2800–3000 m)	*Wei Z. P. 5554 (b), 5564 (b)*	Zhang, 1982
De qin, Yunnan Province, China	–	Gao & Cao, 2000
Da guan, Yunnan Province, China	*Li X. J. 4265*	KUN
Mt. Wuyi, Fujian Province, China	*Li D. K. 11604*	SHM
Mt. Wuyi, Fujian Province, China	*Li D. K. & Gao C. H. 11614, 14079, 14141*	SHM
Bai shanzu Natural Reserve, Zhejiang Province, China (alt. 1600 m)	*Zhu R. L. 90529*	HSNU

Müller, 1951–1958; Schuster, 1966) or Trichocoleaceae (Mizutani & Hattori, 1969). Schuster (1972) proposed a subfamily rank, Trichocoleopsidoideae, to include *Trichocoleopsis* under the family Lepidolaenaceae, and later another subfamily, Neotrichocoleoideae, to include *Neotrichocolea* (Schuster, 1979). Grolle (1983) followed these treatments, but because the name of subfamily Trichocoleopsidoideae is illegitimate in nomenclature, he used Trichocoleopsoideae instead. Furuki and Mizutani (1994) placed the two genera in Lepidolaenaceae and kept *Trichocoleopsis* in subfamily Trichocoleopsoideae, but they moved *Neotrichocolea* to subfamily Lepidolaenoideae.

Based on differences in capsule wall anatomy and other structures between the *Trichocoleopsis–Neotrichocolea* group and Lepidolaenaceae, Inoue (1974) proposed a new family, consisting of the two genera, called Neotrichocoleaceae, which was accepted by Schuster (1984). The family Neotrichocoleaceae was also recognized in the recent liverwort classification systems (Crandall-Stotler & Stotler, 2000; Frey & Stech, 2005; He-Nygrén et al., 2006), but the circumscription of the family has been disputed. Frey and Stech (2005) and He-Nygrén et al. (2006) did not list the members of Neotrichocoleaceae, whereas Crandall-Stotler and Stotler (2000) suggested that the family should only include *Neotrichocolea*, and *Trichocoleopsis* should be transferred back to Lepidolaenaceae.

Molecular data ranging from a single gene to multiple genes have been used to resolve the

relationships of liverworts (e.g., Capesius & Bopp, 1997; Lewis et al., 1997; Stech et al., 2000; Wheeler, 2000; Boisselier-Dubayle et al., 2002; Davis, 2004; Forrest & Crandall-Stotler, 2004, 2005; He-Nygrén et al., 2004, 2006; Crandall-Stotler et al., 2005; Heinrichs et al., 2005; Forrest et al., 2006; Hentschel et al., 2006). These studies represent important progress in understanding the phylogenetic relationships of liverworts at different levels. These studies also provide a useful instrument for resolving the systematic positions of previously unsampled taxa whose placements are controversial or uncertain when based solely on morphology.

Among the above studies, Davis (2004), He-Nygrén et al. (2004, 2006), Heinrichs et al. (2005), Forrest et al. (2006), and Hentschel et al. (2006) sampled *Neotrichocolea*. All molecular analyses indicated that *Neotrichocolea* was closely related to *Ptilidium*; however, the position of this *Ptilidium–Neotrichocolea* clade within the leafy liverworts was ambiguous. In these molecular studies, leafy liverworts are divided into two major clades, which were named "Leafy I" and "Leafy II" by Davis (2004). The *Ptilidium–Neotrichocolea* clade has been resolved in "Leafy I" or "Leafy II" by different studies, but no results have received strong support. Until now, *Trichocoleopsis* has not been included in any molecular study.

In this study, we generated sequence data from four chloroplast genes (small subunit [SSU], large subunit [LSU], *atpB*, and *rbcL*) for *Trichocoleopsis* and *Neotrichocolea*, and two nuclear ribosomal (18S and 26S), one mitochondrial (*nad5*), and two chloroplast (*psbA* and *rps4*) genes for *Trichocoleopsis*. Two different data sets were constructed with different taxon and character sampling schemes. Using these data, we aim to: (1) determine the position of *Trichocoleopsis* within the leafy liverworts, and (2) clarify the relationship between *Trichocoleopsis* and *Neotrichocolea*.

Materials and Methods

Taxon Sampling

The majority of taxa sampled for this study were derived from the recent four-gene analysis reported by Davis (2004) and the six-gene analysis by Qiu et al. (2006). *Trichocoleopsis sacculata* was collected by Liu Yang (collection number *4170*) in 2005 from Mt. Huang, Anhui Province, China (Table 1), and *Neotrichocolea bisseti* by He Lin (collection number *040522*) in 2004 from Mt. Fanjing, Guizhou Province, China (Table 1). The voucher specimens have been deposited in the herbarium of the Institute of Botany, the Chinese Academy of Sciences, Beijing (PE). This study obtained four new sequences for *N. bisseti*: chloroplast SSU ribosomal DNA (rDNA) (GenBank accession number EF460698), LSU rDNA (EF460696), *atpB* (EF460694), and *rbcL* (EF460702); and nine new sequences for *T. sacculata*: chloroplast SSU rDNA (EF460697), LSU rDNA (EF460695), *atpB* (EF460693), *rbcL* (EF460701), *rps4* (EF460703), and *psbA* (EF460700), nuclear SSU (18S) rDNA (EF460691) and LSU (26S) rDNA (EF460692), and mitochondrial *nad5* (EF460699). For the four-gene data set, 57 taxa representing 57 genera in 34 families of liverworts were included. The sampling came mostly from Davis (2004) and focused on the leafy liverworts. For the nine-gene data set, including 24 genera of 19 families, the strategy was to further stabilize the tree topology as sequence data increased. Although taxon density of this data set was lower than that of the four-gene data set, all major lineages of leafy liverworts were sampled. According to previous studies (Davis, 2004; He-Nygrén et al., 2004, 2006; Heinrichs et al., 2005; Forrest et al., 2006), six genera from simple thalloid liverworts were chosen as outgroups in all analyses. In this study, classification followed Crandall-Stotler and Stotler (2000).

Molecular and Phylogenetic Analyses

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing protocols followed Davis (2004) and Liu et al. (2005). All alignments were made using CLUSTAL X 1.83 (Thompson et al., 1997) and then adjusted manually in BioEdit 5.0.9 (Hall, 1999). By comparing the alignments made by Davis (2004) and our own, ambiguous positions (mainly in the *nad5* and 26S) were excluded from phylogenetic analyses. Because we were unable to sequence the 18S gene from *Neotrichocolea*, despite repeated attempts, this sequence was coded as missing data. The resulting matrices have been deposited in TreeBASE (<http://www.treebase.org>; accession number SN3273).

Phylogenetic analyses for each matrix were carried out using maximum parsimony (MP) and maximum likelihood (ML) methods in PAUP* version 4.0b10 (Swofford, 2003) and PHYML version 2.4.3 (Guindon & Gascuel, 2003), respectively. For MP analyses, heuristic searches were conducted with 1000 replicates of each 100 random sequence addition, tree bisection-reconnection (TBR) branch swapping, MulTrees in effect. All characters were unordered and equally weighted, and gaps were coded as missing data. To assess node support, bootstrap analyses (Felsenstein, 1985) were performed using 1000 heuristic search replicates as described above. Likelihood analysis was performed using a general time reversible (GTR) substitution model with invariant sites and additional among-site rate variation

Table 2. Comparison of 57-taxon and 24-taxon MP tree statistics. CI, consistency index; RI, retention index.

	Total characters	Variable characters	Variable informative sites (%)	Trees	Tree length	CI	RI
57 Taxa							
Nuclear only	1077	244	130 (12.1%)	510	686	0.484	0.650
Mitochondrial only	1125	479	241 (21.4%)	12,360	946	0.685	0.687
Chloroplast only	1764	819	660 (37.4%)	4	3756	0.319	0.521
4-Gene combined	3966	1497	1028 (25.9%)	4	5356	0.386	0.549
24 Taxa							
Nuclear only	2391	356	132 (5.5%)	9	672	0.641	0.516
Mitochondrial only	1052	318	130 (12.4%)	14,445	499	0.741	0.584
Chloroplast only	6619	1891	1277 (19.3%)	1	5359	0.477	0.422
9-Gene combined	10,062	2565	1539 (15.3%)	3	6586	0.509	0.430

modeled as a discrete gamma distribution (Yang, 1994). ML parameter values were then optimized, with a BIONJ tree as a starting point (Gascuel, 1997) with the appropriate parameters. Nodal robustness on the ML tree was estimated by the nonparametric bootstrap (1000 replicates).

Results

Four-gene analysis

This data set included nuclear 26S (1077 sites), chloroplast *psbA* (1171 sites) and *rps4* (593 sites), and mitochondrial *nad5* (1125 sites) genes for 57 taxa. Of the total 1028 informative characters, 64.2% and 23.4% derived from the chloroplast and the mitochondrial gene partitions, respectively. MP analysis resulted in four maximally parsimonious trees (MPTs) of length 5356. The consistency index (CI) and retention index (RI) values are consistently higher for mitochondrial genes than for the nuclear or chloroplast genes (Table 2). Topologies resolved by MP and ML analyses are mostly congruent (Fig. 2).

Leafy liverworts are recognized as monophyletic with strong support (97%^{ML}; 92%^{MP}). ML analyses subdivided leafy liverworts into two major clades, "Leafy I" (70%) and "Leafy II" (76%), but the "Leafy I" clade is not found in the strict consensus of four MPTs. In all analyses, *Trichocoleopsis* and *Neotrichocolea* formed a clade (71%^{ML}; 63%^{MP}) sister to *Ptilidium* with strong support. The *Ptilidium*–(*Trichocoleopsis*–*Neotrichocolea*) clade was resolved in "Leafy II" and sister to the remaining taxa (76%^{ML}; 70%^{MP}).

Nine-gene analysis

This data set contained nuclear 26S (1061 sites) and 18S (1330 sites); chloroplast SSU (892 sites), LSU (2162 sites), *atpB* (972 sites), *rbcL* (835 sites), *rps4* (590 sites), and *psbA* (1168 sites); and mitochondrial *nad5* (1052 sites) genes for 24 taxa. Of the total 1539 informative characters, 83.0% and 8.4% derive from the chloroplast and the mitochondrial gene partitions, respectively (Table 2). MP analysis recovered three MPTs of length 6586. The ML tree was largely congruent with the MPTs (Fig. 3). Although taxon sampling was more limited compared with the four-gene data set, the results were similar to the four-gene analysis. *Trichocoleopsis* and *Neotrichocolea* formed a clade (81%^{ML}; 84%^{MP}), sister to *Ptilidium* with strong support, but the *Ptilidium*–(*Trichocoleopsis*–*Neotrichocolea*) clade was identified as sister to "Leafy I" in ML analysis without significant support (50%). The *Ptilidium*–(*Trichocoleopsis*–*Neotrichocolea*) clade was again resolved in "Leafy II" by MP analysis, also without significant support (55%).

Discussion

Trichocoleopsis and *Neotrichocolea* are not members of Trichocoleaceae and Lepidolaenaceae

Some authors (e.g., Nakai, 1943; Müller, 1951–1958; Schuster, 1957; Mizutani & Hattori, 1969; Mizutani, 1972) placed *Trichocoleopsis* and/or *Neotrichocolea* in Trichocoleaceae, based mainly on the presence of a coelocaule. However, as pointed out by Schuster (1966, 1972), there are numerous characters suggesting the remoteness of the relationship between them (Table 3). For example, the former two genera have a coelocaule with simple paraphyllia (usually branched in Trichocoleaceae), incubous leaves (succubous in Trichocoleaceae), relatively reduced underleaves (similar to the lateral leaves in Trichocoleaceae), freely developed rhizoids (usually lacking in Trichocoleaceae), water sacs (absent in Trichocoleaceae), unelongated leaf cells, collenchyma, smooth cilia cells (striolate-papillose in Trichocoleaceae), and relatively large spores (40–55 μm in *Trichocoleopsis* and *Neotrichocolea* but 13–20 μm in Trichocoleaceae). In fact, Asakawa et al. (1981b) hypothesized that *Trichocoleopsis* and *Neotrichocolea* had a distant

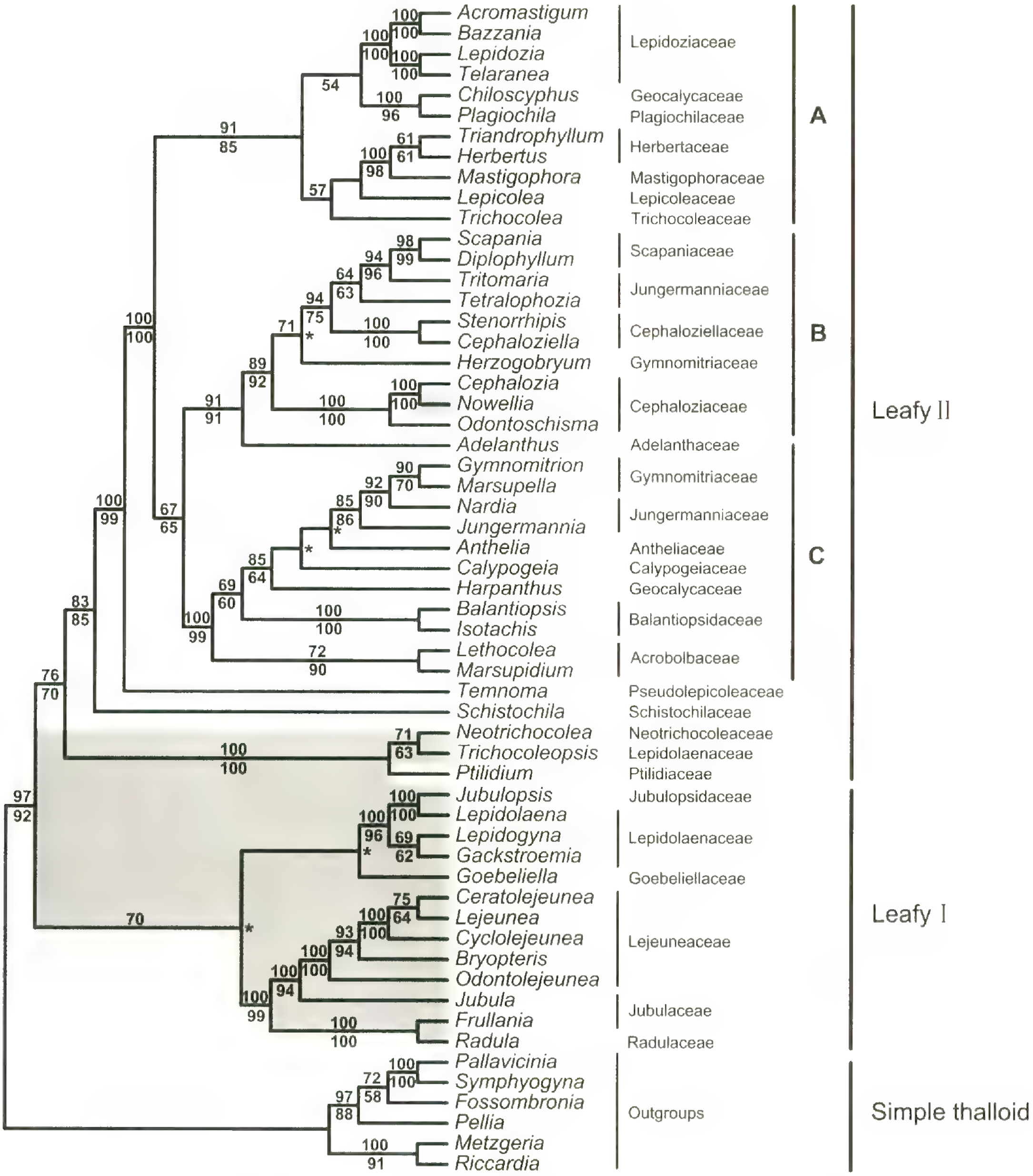

Figure 2. ML tree inferred from the four-gene data set. Numbers above and below branches are ML and MP bootstrap percentages (≥ 50%). Asterisks indicate the nodes not found in the strict consensus of MPTs. Shading represents those genera having leaf water sacs. Higher family names follow the classification of Crandall-Stotler and Stotler (2000). The circumscription of the Leafy I and II liverworts and clades A, B, and C beside the tree are based on Davis (2004).

relationship with *Trichocolea* Dumort., because their chemical components are quite different. In the present molecular analyses, Trichocoleaceae was resolved as a member of the strongly supported clade A in "Leafy II," congruent with previous studies (e.g., Davis, 2004; He-Nygrén et al., 2004, 2006; Heinrichs et al., 2005; Forrest et al., 2006). It is clear that the two endemic genera and Trichocoleaceae belong to two remote clades, respectively (Figs. 2, 3).

In modern classification of liverworts, the placement of *Trichocoleopsis* and *Neotrichocolea* in Lepidolaenaceae was also widely accepted (Schuster, 1972, 1979; Grolle, 1983; Furuki & Mizutani, 1994; Cao et al. 2006; Yamada & Iwatsuki, 2006). However, in this study, Lepidolaenaceae, together with Jubulopsidaceae, formed a well-supported clade (100%^{ML}; 96%^{MP}) (Fig. 2), which was consistent with the results of previous molecular studies (Davis, 2004; He-

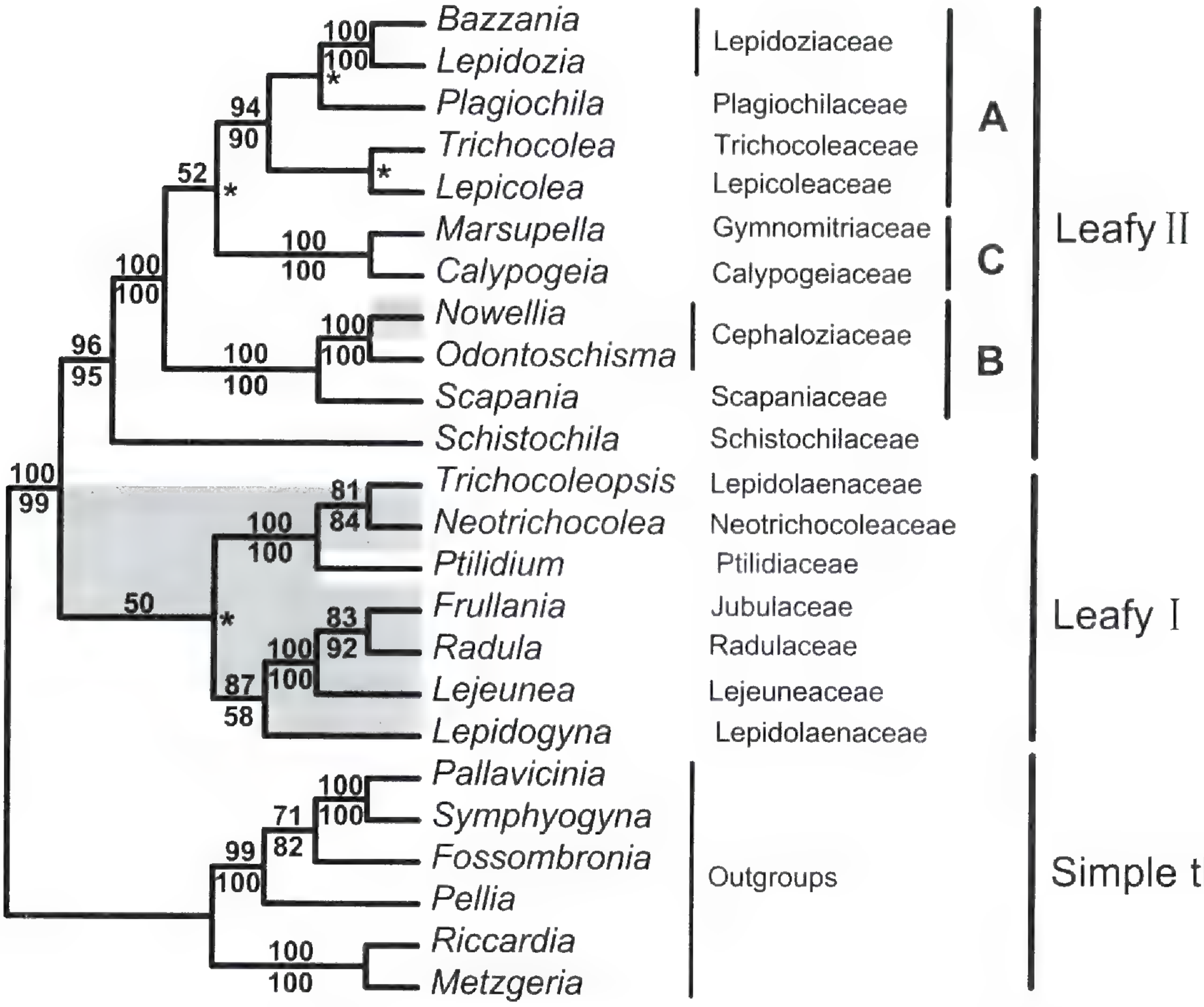

Figure 3. ML tree inferred from the nine-gene data set. Numbers above and below branches are ML and MP bootstrap percentages (≥ 50%). Asterisks indicate the nodes not found in the strict consensus of MPTs. Shading represents those genera having leaf water sacs. Higher family names follow the classification of Crandall-Stotler and Stotler (2000). The circumscription of the Leafy I and II liverworts and clades A, B, and C beside the tree are based on Davis (2004).

Nygrén et al., 2004, 2006; Heinrichs et al., 2005). In the aligned matrix of the *nad5* sequences, there are several unique insertions ("GCACGA" insertion at 740–745, "CAC" at 992–994, and "AAGA" or "AATA" at 1010–1013) shared by Lepidolaenaceae and Jubulopsidaceae, but they are not found in *Trichocoleopsis*, *Neotrichocolea*, or other taxa, which provides additional evidence for the distant relationship between them.

Initially, *Trichocoleopsis* and *Neotrichocolea* were placed in Lepidolaenaceae (Schuster 1966, 1979), based mainly on the endosporic development of the protonema and bearing water sacs. However, there are remarkable differences between the two genera and Lepidolaenaceae. In Lepidolaenaceae, the interior cells of the capsule wall have I- or U-shaped secondary thickenings (Grolle, 1967), but they are extremely thin walled in *Trichocoleopsis* and *Neotrichocolea*. Additionally, water sacs occur both on lateral leaves and underleaves in Lepidolaenaceae, but only on lateral leaves in *Trichocoleopsis* and *Neotrichocolea*. In Lepidolaenaceae, the water sac is derived from the middle lobe of the trifid leaf; by contrast, this structure originates from the ventralmost lobe in *Trichocoleopsis* and *Neotrichocolea*. Additionally, Lepidolaenaceae is distributed in Gondwanian regions, whereas *Trichocoleopsis* and *Neotrichocolea* are endemic to eastern Asia. If the two genera were considered members of Lepidolaenaceae, this disjunction would be so rare that Schuster (1983), under this assumption, once called *Trichocoleopsis* and *Neotrichocolea* "the most striking hepatic endemics in eastern Asia."

THE RELATIONSHIP BETWEEN *TRICHOCOLEOPSIS* AND *NEOTRICHOCOLEA*

Recent molecular studies all support that *Neotrichocolea* is closely related to *Ptilidium* (Davis, 2004; He-Nygrén et al., 2004, 2006; Heinrichs et al., 2005; Forrest et al., 2006; Hentschel et al., 2006). Our results showed that *Neotrichocolea* had a closer relationship with *Trichocoleopsis* than with *Ptilidium*, although the three genera formed a strongly supported monophyletic group (Figs. 2, 3). Inoue (1974) indicated that *Trichocoleopsis* and *Neotrichocolea* were

Table 3. Comparison of morphological characters in *Trichocoleopsis*, *Neotrichocolea*, and other related genera as described in detail by Schuster (1966, 1972), Grolle (1967), and Inoue (1974). NA, not available.

Character	*Trichocoleopsis*	*Neotrichocolea*	*Ptilidium*	*Lepidolaena* Dumort.	*Trichocolea*
Branching types	1 to 2 pinnately branched, *Frullania* type	3 to 4 pinnately branched, *Frullania* type	1 to 3 pinnately branched, *Frullania* type	2 to 4 pinnately branched, *Frullania* type	1 to 3 pinnately branched, *Frullania* type
Subfloral innovation	present	present	absent	present	present
Leaf insertion pattern	incubous	incubous	transverse (upper end incubous)	incubous	succubous
Leaf and underleaf	lobed, margins ciliate, cilia cells smooth, underleaf smaller than lateral leaf	lobed, both margin and dorsal surface of leaf ciliate, cilia cells smooth, underleaf smaller than lateral leaf	lobed, margins ciliate, cilia cells smooth, underleaf smaller than lateral leaf	not lobed, margins without or with short cilia, cilia cells smooth, underleaf smaller than lateral leaf	strongly lobed, margins ciliate, cilia cells cuticle striolate-papillose, underleaf similar to lateral leaf
Water sacs	present	present	absent	present	absent
Leaf cells and oil bodies	irregularly polygonal, with small trigones, oil bodies granular botryoidal	round rectangular, with weak trigones, oil bodies granular botryoidal	irregularly polygonal, with bulging trigones, oil bodies nearly homogeneous	irregularly polygonal, with bulging trigones, oil bodies nearly homogeneous	rectangular, without obvious trigones, oil bodies nearly homogeneous
Rhizoids	present	present	absent	NA	absent
Perianth	absent, having coelocaules	absent, having coelocaules	present	absent, having coelocaules	absent, having coelocaules
Capsule	apex rounded, epidermal cells with secondary thickenings at corners, interior cells thin walled, easily removed	apex rounded, epidermal cells with secondary thickenings at corners, inner cells thin walled, easily removed	apex beaked, epidermal cells with nodular thickenings, inner cells thinner with semicircular thickenings	apex beaked, epidermal and inner cells equally sized, all have I- or U-shaped secondary thickenings	apex rounded, epidermal cells hyaline, without secondary thickenings, inner cells with distinct secondary thickenings
Spore diameter	48–55 μm	40–48 μm	25–27 μm	50–80 μm	13–20 μm
Sporeling type	endosporous, *Trichocoleopsis* type	endosporous	exosporous, *Nardia* type	endosporous, *Frullania* type	exosporous, *Nardia* type

closely allied to each other. Indeed, the two genera are similar in many morphological characters, such as the lack of a perianth, the presence of a paraphyllose, massive coelocaule, and the interior cells of the capsule wall being extremely thin walled and easily removed. Additionally, they all possess incubous leaves, with cilia on the lobed leaf margins, water sacs, and semblable oil bodies (Table 3). Moreover, a similar distribution pattern (Fig. 1) and habitat (Chen & Wu, 1965) are shared by the two genera. However, as described by Hattori (1947), there are enough differences to separate them at the generic level: cilia occur only on leaf margins in *Trichocoleopsis* but both on the margin and dorsal surface of leaves in *Neotrichocolea*; and water sacs are present on stem and branch leaves of *Trichocoleopsis* but only on branch leaves in *Neotrichocolea*. Moreover, the development and shape of water sacs are distinct in the two genera.

Water sacs are widely observed in certain members of "Leafy I" (see shading in Figs. 2, 3) and were regarded as a device for facilitating water storage (Schuster, 1984). Their shapes can be cup-like (in *Frullania* Raddi), horn-shaped (in *Goebeliella* Steph.), or even specialized by having a trap-like structure in *Colura* (Dumort.) Dumort. and *Pleurozia* Dumort., which are considered to have a zoophagy function (Hess et al., 2005). According to development in dissection, saccate lobules are divided into two kinds: the "*Lejeunea* Lib. type" and "*Frullania* type." A *Lejeunea*-type water sac comes from an inrolling of the free margin of the lobule against the back side of the lobe and is formed from both lobe and lobule tissue. A *Frullania*-type water sac, in contrast, involves the development of a secondary meristematic region in the midzone of the lobule. At an early stage, the lobule inrolls to the dorsal lobe. Afterward, continued divisions cause the upper part of the lobule to balloon or inflate down upon itself (Crandall-Stotler & Guerke, 1980). The opening of this kind of lobule faces away from the apex of the stem.

Trichocoleopsis and *Neotrichocolea* were described as having *Lejeunea*-type (Shuster, 1972) and *Frullania*-type (Evans, 1905) water sacs, respectively. In *Trichocoleopsis*, the free margin of the lobule is deeply inrolled, and it can be drawn open like a spring. This type of deep inrolling is also found in many Lejeuneaceae (such as *Neurolejeunea* (Spruce) Schiffn., *Mastigolejeunea* (Spruce) Schiffn., and *Siphonolejeunea* Herzog). As in other *Lejeunea*-type lobules, a *Trichocoleopsis* lobule is inserted in a U line on the stem, so the whole leaf has a J-shaped insertion. In *Neotrichocolea*, the *Frullania*-type water sac is a slipper-like structure, opening parallel to the stem, but is not cup-like as in *Frullania*. It is remarkable that genera with such different lobule development patterns are so closely related. *Trichocoleopsis* and *Neotrichocolea* do share unique lobule position: water sacs of both come from the ventral-most lobe of the trifid leaf. In contrast, other *Lejeunea*-type and *Frullania*-type water sacs are typically derived from the middle lobe of the trifid leaf. The third lobe can convert to a stylus, as in certain species of *Frullania* and Lepidolaenaceae, or be reduced to a slime papilla as in Lejeuneaceae and *Jubula* Dumort. (Schuster, 1972).

AFFINITIES OF *TRICHOCOLEOPSIS* AND *NEOTRICHOCOLEA*

In the present study, analyses of the four- and nine-gene data sets all strongly support *Ptilidium* as sister to *Trichocoleopsis* and *Neotrichocolea*. In the alignment of *nad5* sequences, there was an exclusive "CAT" insertion at the position of 593–595 shared by *Neotrichocolea*, *Trichocoleopsis*, and *Ptilidium*, which further supported the close relationship between them. *Trichocoleopsis* was described as having a special endosporic sporeling type (*Trichocoleopsis* type [Inoue, 1956]), which is different from the exosporous *Nardia* Gray type of *Ptilidium*. However, Inoue (1963) described that in *T. sacculata*, the early stage of development of the leafy shoot was essentially the same as that of *Ptilidium*, indicating a potential relationship between them. Additionally, a unique furanosesquiterpene, deoxopinguisone, and its derivatives were shared by *Ptilidium* and *Trichocoleopsis* (Asakawa et al., 1981a).

Ptilidiaceae, once a portmanteau family of liverworts, was suggested to contain 15 genera by Evans (1939). Over subsequent years, most of these were gradually removed from the family, until only *Ptilidium* remained (Schuster, 1979, 1984; Grolle, 1983; Crandall-Stotler & Stotler, 2000). *Trichocoleopsis* and *Neotrichocolea* were previously placed in Ptilidiaceae (Evans, 1939; Hattori, 1947; Schuster, 1966; Mizutani & Hattori, 1969). Some key differences in morphology between the *Trichocoleopsis–Neotrichocolea* group and Ptilidiaceae precipitated their removal (Table 3). For example, *Ptilidium* has a well-developed perianth, which was considered the most important difference between *Ptilidium* and the *Trichocoleopsis–Neotrichocolea* group, and also between Trichocoleaceae and Lepidolaenaceae (Schuster, 1972; Inoue, 1974). Additionally, *Trichocoleopsis* and *Neotrichocolea* have water sacs on their leaves (absent in *Ptilidium*), subfloral innovations and rhizoids (absent in *Ptilidium*), rounded capsules (beaked in *Ptilidium*), much larger spores, and different oil body types than *Ptilidium* (Table 3).

Phytogeographically, *Trichocoleopsis* and *Neotrichocolea* are endemic to East Asia, but *Ptilidium* has a

holarctic distribution pattern. Schuster (1983) presumed that *Trichocoleopsis*, *Neotrichocolea*, and some other eastern Asian endemic liverwort genera, such as *Nipponolejeunea* Hatt., *Scaphophyllum* Inoue, and *Hattoria* R. M. Schust., might have had a wider range before and formed their modern distribution patterns because of extinctions in other regions. For example, the two-species genus *Nipponolejeunea* is endemic to northeastern Asia, but another amber fossil species from the Oligocene was found in Europe (Grolle, 1981). Such endemism seemed to be a relict. Thus, excessive extinctions of taxa between *Ptilidium* and the *Trichocoleopsis–Neotrichocolea* group might have occurred, which could explain the morphological gap between them. Indeed, *Trichocoleopsis* and *Neotrichocolea* may retain many primitive characters compared to *Ptilidium*, such as possessing water sacs and endosporous protonemata.

POSITION OF THE *PTILIDIUM*–(*TRICHOCOLEOPSIS*–*NEOTRICHOCOLEA*) CLADE

Based on different sampling density (taxon and gene) and analysis methods, previous molecular studies (e.g., Davis, 2004; He-Nygrén et al., 2004, 2006; Heinrichs et al., 2005; Forrest et al., 2006) have identified *Ptilidium* and/or *Neotrichocolea* close to "Leafy I" or "Leafy II," or as a part of basal leafy polytomy. However, none of these studies obtained support for these placements. Recently, Hendry et al. (2007) reported that the New Zealand endemic genus *Herzogianthus* R. M. Schust. seemed to be related to the *Ptilidium–Neotrichocolea* clade, but the position of this clade and the endemic genus within liverworts remained unresolved. According to our analyses, the *Ptilidium*–(*Trichocoleopsis–Neotrichocolea*) clade was resolved with moderate support as a member of "Leafy II" based on the ML and MP analyses of the four-gene data matrix, and was placed together with "Leafy I" in the ML analyses of the nine-gene matrix without significant support. The addition of the genus *Trichocoleopsis* did not provide enough new information to definitively resolve the position of the *Ptilidium*–(*Trichocoleopsis–Neotrichocolea*) clade.

This problem, and its implications for understanding morphological evolution in the leafy liverworts, have been discussed in detail in recent studies (see Forrest et al., 2006). Both Heinrichs et al. (2005) and He-Nygrén et al. (2006) suggested that "Leafy I" and the "*Ptilidium* clade" should be grouped in the order Porellales, even though this relationship was not supported in their molecular analyses. Various shared morphological characters were cited to support this viewpoint, such as the mutual *Frullania* branching type, lack of ventral branching, incubous leaves, endogenous spore germination (however, *Ptilidium* lacks this trait), and some chemical components.

Conclusions

Molecular and morphological evidence showed that *Trichocoleopsis* and *Neotrichocolea* represent a monophyletic group. The family Neotrichocoleaceae, which includes the two genera as suggested by Inoue (1974), should be recognized. The family is characterized by having incubous leaves with cilia on the lobed leaf margins, water sacs, a paraphyllose coelocaule, and extremely thin-walled interior capsule cells. Molecular results indicated that Neotrichocoleaceae was allied to Ptilidiaceae, but except for the similar ciliate and lobed leaves, there are almost no known synapomorphies shared by the two families. On the contrary, Neotrichocoleaceae differ from Ptilidiaceae in many characters. Thus, merging the three genera into one family is premature based on the current evidence. The exact position of the Neotrichocoleaceae–Ptilidiaceae clade remains unresolved in the present study. A better sampling of both sequence data and taxa is required before the problem can be resolved.

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A TAXONOMIC REVISION OF THE SOUTH AFRICAN ENDEMIC GENUS *ARCTOPUS* (APIACEAE, SANICULOIDEAE)[1]

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ABSTRACT

The genus *Arctopus* L. is revised. It is an anomalous member of the family Apiaceae, with several unusual morphological characters such as the prostrate spiny leaves, thick resinous tuberous roots, sessile female flowers with a homochlamydeous perianth, and pseudanthia formed by distinctive bracteoles. The three species of *Arctopus* were found to differ mainly in their reproductive morphologies, being easily distinguishable by the large involucral bracteoles that surround the female pseudanthia. The anatomy of the petiole as well as the morphology of the inflexed spines and leaf margins were also found to be useful diagnostic characters and are illustrated and discussed. Possible phylogenetic relationships were explored using both morphological and DNA sequence data (ITS). A formal taxonomic treatment is presented, including a key to the species, correct nomenclature, typification, descriptions, illustrations, and distribution maps.

Key words: Apiaceae, *Arctopus*, ITS, IUCN Red List, phylogeny, Saniculoideae, taxonomy.

Arctopus L. is endemic to the Cape Floristic Region (CFR) of South Africa and consists of three dioecious species. They are important medicinal plants with a rich ethnobotanical history (Magee et al., 2007). *Arctopus* was described by Linnaeus (1753) in *Species Plantarum*, from figures by Plukenet (1691–1705: tab. 271, fig. 5) and Burman (1738: tab. 1). The name *Arctopus* is derived from Greek, meaning "bear's foot," due to the broad simple leaves that remain appressed to the ground and are armed with large inflexed spines. The genus was originally thought to be monotypic, consisting only of *A. echinatus* L., whose virtue as an important medicinal plant was well known to the Khoisan people (Pappe, 1857). It also became popular among the early Cape Dutch settlers and became known by the vernacular names "sieketroost" or "platdoring" (Smith, 1966). Two additional species, *A. dregei* Sond. and *A. monacanthus* Carmich. ex Sond., were later described by Sonder (1862) in *Flora Capensis*. Wolff last revised the genus in 1913 but reported male material of *A. monacanthus* as unknown. Because the species circumscriptions were based solely on female material, the identification of male plants has been difficult, resulting in much confusion.

This paper is aimed at presenting a complete revision of the genus, including the circumscription, nomenclature, typification, formal descriptions, and illustrations of diagnostic characters for the three species that are recognized. A key to aid identification of female and, for the first time, male non-flowering material is provided. The known distribution of each species is recorded. Morphological, anatomical, and sequence data (ITS) from *Arctopus* are also evaluated in order to explore possible phylogenetic relationships at both generic and species level.

TAXONOMIC POSITION

Arctopus is an anomalous genus of geophytes belonging to the family Apiaceae. There have been differences of opinion as to the correct taxonomic position of the genus within the family. Drude (1897–1898) divided Apiaceae into the three subfamilies Apiodeae, Saniculoideae, and Hydrocotyloideae. He placed *Arctopus* in the subfamily Saniculoideae based on the presence of toothed leaves, surface outgrowths on the mericarps, and intrajugal oil ducts. In Wolff's (1913) treatment, *Arctopus* was also placed in Saniculoideae. However, both Froebe (1964) and Magin (1980), in their studies of the inflorescence structure of the Saniculoideae, found that *Arctopus* shares many characteristics with the Hydrocotyloideae. Pimenov and Leonov (1993) listed it in the

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Table 1. Material of *Arctopus* species used for anatomical studies (all material kept at JRAU). f, flower; ifr, immature fruit; l, lamina; md, midrib; mfr, mature fruit; p, petiole.

Taxon	Voucher specimens	Locality	Plant parts studied
A. dregei	*Magee & Boatwright 2*	Rondeberg	p, l, md, mfr
	Magee & Boatwright 5	Darling	p, md, f, mfr
	Magee & Boatwright 31	Malmesbury	p, mfr
A. echinatus	*Magee & Boatwright 4*	Rondeberg	p, md
	Magee & Boatwright 6	Yzerfontein	p, l, md, mfr
	Van Wyk & Viljoen 3715	Oorlogskloof	p, l, md
	Van Wyk 4128a	Nieuwoudtville	md, f
	Van Wyk s.n., 14 Oct. 1993	Arendskraal	p, mfr
	Winter et al. 170	Du Toitskloof	f
A. monacanthus	*Magee & Boatwright 34*	Pakhuis Pass	p, mfr
	Van Wyk 3594	Piketberg	p, ifr
	Van Wyk 4128b	Nieuwoudtville	md
	Van Wyk 4141a	Elandskloof	p, l, md, f, mfr
	Van Wyk 4161a	Gifberg	p, md
	Van Wyk s.n., 12 July 1997	Jonaskop	p, md
	Van Wyk, Winter & Tilney s.n., 8 Oct. 1993	McGregor Rd.	p

subfamily Hydrocotyloideae but were uncertain about the tribal affinity (listed under incertae sedis). However, recent molecular data (Plunkett & Lowry, 2001; Chandler & Plunkett, 2004; Plunkett et al., 2004) show Hydrocotyloideae to be polyphyletic. A new subdivision of the family into Apioideae, Saniculoideae, Azorelloideae, and Mackinlayoideae has been proposed (Plunkett et al., 2004), in which a close relationship between *Arctopus* and the Saniculoideae is suggested. Liu et al. (2003), in their evaluation of the fruit structure of Saniculoideae, proposed a broader saniculoid concept that includes *Arctopus* and some other anomalous African genera (viz. *Lichtensteinia* Cham. & Schltdl., *Polemanniopsis* B. L. Burtt, and *Steganotaenia* Hochst.). Calviño and Downie (2007), using chloroplast sequence data (*trnQ-trnK* 5′: exon region) to assess the circumscription of the subfamily, proposed two tribes within Saniculoideae, namely a redefined Saniculeae and a newly described Steganotaenieae (comprising *Polemanniopsis* and *Steganotaenia*). In this analysis, *A. echinatus* is placed within Saniculeae as a sister group to *Eryngium* L., *Sanicula* L., *Hacquetia* Neck. ex DC., *Petagnaea* T. Caruel, *Astrantia* L., and *Actinolema* Fenzl, with *Alepidea* D. Delaroche successively sister and the most basally diverging lineage within the tribe.

Materials and Methods

The three species were studied in situ and observed throughout much of their known distributional range. Herbarium material from BM, BOL, JRAU, K, NBG, PRE, S, and SAM was studied (abbreviations follow Holmgren et al., 1990). From this material, together with geographical information from Leistner and Morris (1976), the recorded distributions of all the species were carefully verified and mapped. The conservation status of each species was assessed using IUCN Red List criteria (IUCN, 2001).

For anatomical procedures, leaf (petiole, lamina, and midrib) and fruit material were fixed in formalin, acetic acid, and alcohol (FAA) for at least 24 hr. and then treated according to the method of Feder and O'Brien (1968) for embedding in glycol methacrylate (GMA), but modified so that the material in the first two changes of GMA was infiltrated for a minimum of 24 hr., followed by a final infiltration of five days. Staining was done according to the periodic acid Schiff/toluidine blue (PAS/TB) staining method (Feder & O'Brien, 1968). A list of voucher specimens for the anatomical study is given in Table 1. Drawings, all done by the first author, were made with the aid of a camera lucida attachment on a Zeiss (Göttingen, Germany) compound microscope.

Total DNA was extracted from 0.5–1.0 g of fresh leaf material following the CTAB method of Doyle and Doyle (1987). The extracts were cleaned and concentrated in QIAquick silica columns according to the manufacturer's protocol (Qiagen Inc., Hilden, Germany). Genera of the Saniculoideae belonging to both tribes Saniculeae (*Alepidea*, *Astrantia*, *Erygium*, *Hacquetia*, *Petagnaea*, and *Sanicula*) and Steganotaenieae (*Steganotaenia*) were included in the analysis, together with two representative genera of the Apioideae (*Heteromorpha* Cham. & Schltdl. and *Anginon* Raf.). *Hermas villosa* Thunb. was selected as the outgroup based on the sister relationship

Table 2. Data matrix of character states in the genus *Arctopus*, used to construct the cladogram in Figure 4. For details of the characters and polarization of character states, see Appendix 2.

Taxa	Characters and character states														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Alepidea amatymbica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arctopus dregei	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1
Arctopus echinatus	1	1	1	1	1	1	0	0	0	0	1	1	0	0	1
Arctopus monacanthus	1	2	1	0	1	1	1	1	1	1	1	1	0	0	1

between the *Hermas* L. clade and the clade of Apioideae and Saniculoideae found by Calviño et al. (2006). Voucher specimen information (including sources of plant material, GenBank accession numbers, species names, and their author citations) is listed in Appendix 1.

The ITS of nuclear ribosomal DNA was amplified using the Sun et al. (1994) AB101 (5′-ACG AAT TCA TGG TCC GGT GAA GTG TT-3′) and AB102 (5′-TAG AAT TCC CCG GTT CGC TCG CCG TT-3′) primers. Amplified polymerase chain reaction (PCR) products were purified using a QIAquick PCR purification kit (Qiagen Inc.) according to the manufacturer's instructions and directly sequenced on a 3130 *xl* Genetic Analyzer (Applied Biosystems Inc., Foster City, California, U.S.A.) using BigDye Terminator version 3.1 (Applied Biosystems Inc.). Complimentary strands were assembled and edited using Sequencher version 3.1.2 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.), and aligned manually in PAUP* version 4.0b1 (Swofford, 2002), with gaps positioned so as to minimize nucleotide mismatches.

Characters and character states used for the cladistic analysis of morphological data are given in Table 2 and Appendix 2. Character states were polarized using the method of outgroup comparison, with *Alepidea amatymbica* Eckl. & Zeyh. as the outgroup. Where both states were found to co-occur in a single taxon, it was coded for the plesiomorphic state. Phylogenetic analyses were conducted using the parsimony algorithm of PAUP* version 4.0b1. Morphological characters were treated as ordered and equally weighted (Wagner parsimony; Farris, 1970), whereas for the molecular data, all character transformations were treated as equally likely (Fitch parsimony; Fitch, 1971). Tree searches were performed using a heuristic search with 1000 random sequence additions, tree bisection-reconnection (TBR) branch swapping, and the MULPARS option in effect. A limit of 10 trees per replicate was set to reduce the time spent on swapping in each replicate. Internal support was assessed with 1000 bootstrap replicates (Felsenstein, 1985) using TBR swapping and holding 10 trees per replicate. Only values greater than 50% are reported, and the following scale was applied for support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Bayesian analysis was performed on the ITS sequence data, using MRBAYES v. 2.01 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The TrN+G model, indicated to be the best model by MODELTEST v. 3.06 (Posada & Crandall, 1998), was implemented. The analysis was performed with one million generations of Markov chain Monte Carlo and a sampling frequency of 100. The resulting trees were plotted against their likelihoods in order to determine where the likelihoods converge on a maximum value. All the trees before this convergence were discarded as the "burn-in" phase. The remaining trees were imported into PAUP* v. 4.0b1, and a majority rule consensus tree was produced in order to show the posterior probabilities (PP) of all observed bi-partitions (only PPs above 0.5 are reported on the tree in Fig. 4). The following scale was used to evaluate the PPs: 0.50–0.84, low; 0.85–0.94, moderate; 0.95–1.0, strong.

Morphological and Taxonomic Characters

Habit

Arctopus is unlike any other genus found within the Apiaceae. The species are summer deciduous geophytes not exceeding 15 cm in height when in flower. Their broad simple leaves, which arise directly from a tuberous rootstock, are spiny and sprawl out in a rosette, remaining appressed to the ground. The plants are distinctly dioecious, and each sex is immediately apparent when in flower or fruit. The three species show no obvious differences in growth form.

Leaf Shape

The leaves of *Arctopus* species are simple and vary greatly in shape, from ovate to semi-orbicular or rhomboidal (Figs. 5A, B, 7A–F, 9A–C), with only

those coastal populations of *A. echinatus* that occur between Knysna and Hermanus bearing leaves that are pinnatipartite to pinnatifid (Fig. 7D, E). In all the species, the leaves are incised into three segments, each of these being two- or three-lobed. In *A. monacanthus* and *A. echinatus*, the lobes are prominently toothed, giving them a very distinctly dentate appearance (Figs. 7A, C–F, 9A–C), whereas in *A. dregei*, the teeth are inconspicuous and thus the lobes appear crenate (Fig. 5A, B).

LEAF VESTITURE

The teeth on the leaf margin terminate in either flexuose setae (the presumed plesiomorphic state found in *Alepidea*) as in *Arctopus dregei*, or spinose setae as in *A. echinatus* and *A. monacanthus*. Such marginal hairs or setae are a characteristic feature of many genera belonging to the subfamily Saniculoideae (van Wyk & Tilney, 2004). Within *Arctopus*, leaf vestiture was found to be a useful diagnostic character. Vertical spines are usually present in the recesses between the leaf segments. In *A. monacanthus*, these spines are attached to an inflexed laminar tooth (Fig. 9D–I), which is often conspicuously broad with marginal setae almost invariably present. However, in *A. echinatus*, the inflexed laminar tooth is either absent or very narrow and almost always without marginal setae (Fig. 7L–N). Inflexed laminar teeth and vertical spines are very rarely present in *A. dregei* (if present, then small, sparse, and attached to a narrow laminar tooth, without setae). The leaves of *A. monacanthus* are markedly more spinescent than those of *A. echinatus*, with up to six stellate spines in each leaf recess being common. In *A. echinatus*, the spines are often solitary or in threes; however, populations from the Cederberg in the north and around Port Elizabeth in the Eastern Cape have up to four or rarely five stellate spines per recess.

LEAF ANATOMY

While the three species are very similar in their vegetative anatomy, certain characters (as discussed later) have been found to be of diagnostic value, particularly in the field where they are easily observed with a 10× hand lens. This is especially true of *Arctopus echinatus* and *A. monacanthus*, which are often difficult to identify when female flowering or fruiting material is not available. *Arctopus dregei* shows much variability in its vegetative anatomy and often shares similar character states to *A. echinatus* and *A. monacanthus*. Because the macromorphology of this species is relatively distinctive, it is not easily confused, even when flowering or fruiting material is unavailable. The use of anatomical characters to distinguish it is therefore superfluous.

Transverse sections of the lamina were found to be markedly similar in all three species and were therefore of little diagnostic value. The mesophyll is distinctly differentiated into palisade and spongy parenchyma, with the palisade parenchyma comprising about half of the mesophyll thickness in *Arctopus echinatus* and *A. monacanthus*, and less than half in *A. dregei*.

The midrib of the lower third of the leaf, when studied in transverse section, was found to be far more useful (Fig. 1A–F). The extent of sub-epidermal collenchyma and the arrangement of the vascular bundles are diagnostic. Sub-epidermal collenchyma is found on both the adaxial and abaxial surfaces. On the abaxial surface, the collenchyma extends at least halfway along the midrib in *Arctopus monacanthus* (Fig. 1E, F) and, to a lesser extent, in *A. dregei* (Fig. 1A, B) and *A. echinatus* (Fig. 1C, D). At least two large, oppositely arranged vascular bundles can usually be found. Of these, the adaxial bundle is solitary (Fig. 1A–C) or further split dorsiventrally (Fig. 1B–D), that is, into a dorsal and a ventral bundle in *A. echinatus* and in *A. dregei*. In *A. monacanthus*, the adaxial bundle is always collaterally split (Fig. 1E, F), that is, into two lateral bundles, although this has also been found to occur occasionally in *A. dregei* (Fig. 1B). One or two additional smaller adaxial bundles are often found in the midribs of larger leaves in all three species.

The transverse sections of the petiole are similarly diagnostic (Fig. 1G–I). The petioles are adaxially flattened and prominently raised abaxially. They extend laterally into wings, which are always prominently ribbed in *Arctopus dregei* (Fig. 1G). Sub-epidermal collenchyma is present along the abaxial surface in all three species. In *A. echinatus* (Fig. 1H) and *A. monacanthus* (Fig. 1I), collenchyma is also found in the wings, but in *A. monacanthus*, it is continuous, or almost so, with the abaxial collenchyma strand. Large cavities between the vascular bundles can be found in *A. echinatus* (Fig. 1H) and in the late season growth of *A. dregei*, but never in *A. monacanthus*.

FEMALE INFLORESCENCE

The bracteoles of the involucel are of diagnostic value and render a female flowering plant quite unmistakable. They are prominent and accrescent in all three species, giving the umbellule the appearance of a single flower, called a pseudanthium (Figs. 5C–F, 7O–R, 9J–M). This is commonly found in other

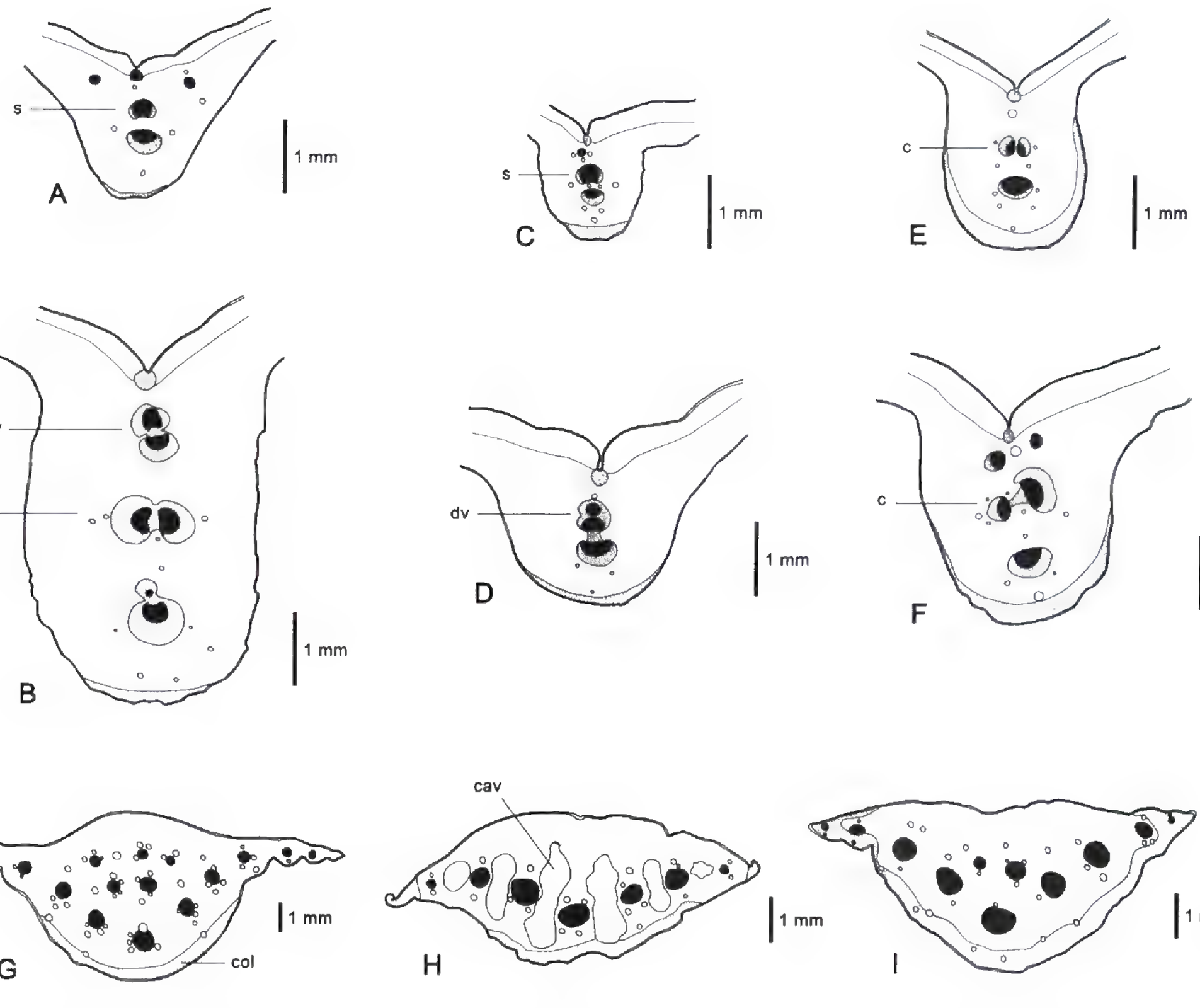

Figure 1. Transverse sections through the midribs (A–F) and petioles (G–I) of *Arctopus* species to illustrate diagnostic anatomical differences. In the midribs, the adaxial vascular bundle can be solitary (C) or dorsiventrally split (D) in *A. echinatus*, collaterally split in *A. monacanthus* (E, F) and a combination of all of these in *A. dregei* (A, B). In the petioles, the presence of collenchyma in the wings of *A. echinatus* (H) and *A. monacanthus* (I), and the markedly ribbed wings in *A. dregei* (G) are characteristic. Cavities between the vascular bundles of healthy, mature leaves are present only in *A. echinatus* (H). —A. *A. dregei* (*Magee & Boatwright 2*, JRAU). —B. *A. dregei* (*Magee & Boatwright 5*, JRAU). —C. *A. echinatus* (*Van Wyk & Viljoen 3715*, JRAU). —D. *A. echinatus* (*Magee & Boatwright 6*, JRAU). —E. *A. monacanthus* (*Van Wyk 4141a*, JRAU). —F. *A. monacanthus* (*Van Wyk s.n., Jonaskop*, JRAU). —G. *A. dregei* (*Magee & Boatwright 2*, JRAU). —H. *A. echinatus* (*Van Wyk s.n., Arendskraal*, JRAU). —I. *A. monacanthus* (*Van Wyk 3594*, JRAU). c, collaterally split adaxial bundle; cav, cavity; col, collenchyma; dv, dorsiventrally split adaxial bundle; s, solitarily adaxial bundle.

members of the Saniculoideae such as *Alepidea*, *Astrantia*, and *Eryngium* (Burtt, 1991).

In *Arctopus*, the pseudanthial bracteoles are fused to the base of the fruit and, as in *Alepidea*, to one another. The bracteoles of *A. dregei* (Fig. 5F) are obovate and involute, with an obtuse spine-tipped apex and one or two inflexed, spinescent hairs along the margin. In *A. monacanthus* (Fig. 9L, M), they become very large, widely obovate and foliose, with an obtuse, spine-tipped apex. Their foliose appearance is enhanced by the prominent reticulate venation. The margin is usually entire, but one to three marginal spines may rarely occur. In *A. echinatus* (Fig. 7R), the bracteoles are ovate, boat-shaped and keeled, with an acute spine-tipped apex plus one or two inflexed and overlapping spines along the margin. They are markedly thickened along the margin and midrib. The pseudanthium of *A. dregei* consists of three to six bracteoles that become white when dry, whereas *A. echinatus* and *A. monacanthus* have either four or five bracteoles that become brown when dry. In *A. monacanthus*, the pseudanthium is brittle and papery at the fruiting stage and tends to break apart easily, so that the bracteoles separate from one another with the ripe fruit still attached to them. In *A. dregei* and *A. echinatus*, the pseudanthium becomes leathery at the fruiting stage and does not break apart easily, so that intact pseudanthia are found with the dying leaves at the end of the growing season (November–December).

FLOWERS

The male flowers are pentamerous with five large petaloid sepals (Figs. 5L, 7H, 9Q) that are linear to oblong and about the same size as the petals. According to Drude (1897–1898), large sepals are commonly found in Saniculoideae (i.e., *Sanicula*, *Hacquetia*, *Actinolema*, and *Eryngium*). There are five oblong to

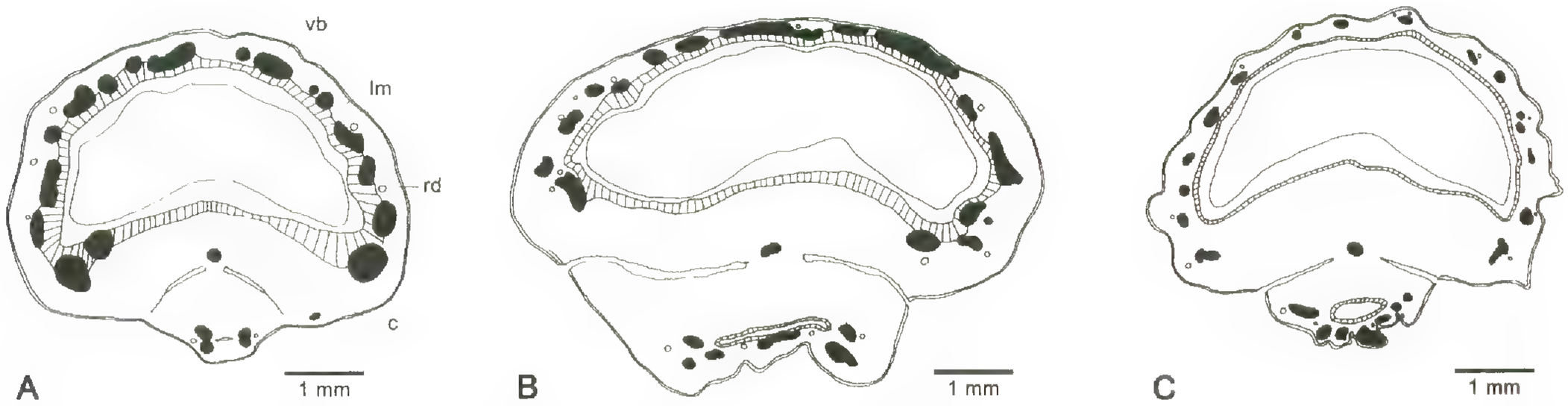

Figure 2. Transverse sections through the pseudo-monocarpellate mature fruit of *Arctopus* species. In all three species, both the single endocarp layer as well as the adjacent mesodermal cells are lignified (a generic synapomorphy). The epidermis remains continuous between both mericarps in *A. dregei* (A), but becomes discontinuous between the two mericarps in *A. echinatus* (B) and *A. monacanthus* (C). —A. *A. dregei* (*Magee & Boatwright 2*, JRAU). —B. *A. echinatus* (*Van Wyk s.n., Arendskraal*, JRAU). —C. *A. monacanthus* (*Van Wyk 4141a*, JRAU). c, carpophore; lm, lignified mesocarp; rd, rib oil duct; vb, vascular bundle.

oblanceolate petals with inflexed tips (Figs. 5M, 7G, 9R, S). On the inner surface, a thin septum joins the basal and apical portions of the inflexed petal, preventing the petals from straightening. The stamens are twice as long as the petals, and the filaments are inflexed at their tips. Although the ovary is absent, a flattened, papillate stylopodium (common to many Saniculoideae) is present along with reduced styles.

The female flowers are sessile (as in *Alepidea*) and tubular (Figs. 5G, 7U, 9O), with a homochlamydeous perianth (a character unique to this genus within the family) consisting of 10 persistent triangular lobes (Figs. 5J, 7V, 9N). Due to the typically pentamerous nature of the male flowers and the almost petaloid sepals, these perianth lobes are here interpreted as five sepals and five sepaloid petals in the female flower, but further ontogenetic studies are required to resolve the homology of these peculiar structures. Stamens are absent and the stylopodium is papillate, cushion-shaped, and swollen so that it almost completely envelops the two style bases. The styles are conical in shape and longer than the sepals. The ovary is bilocular.

FRUIT MORPHOLOGY AND ANATOMY

The fruit of *Arctopus* are unusual in Apiaceae because they become pseudo-monocarpellate with the abortion of one of the two mericarps early in fruit development (Fig. 2). However, in some populations of *A. echinatus* around Vanrhynsdorp and in the Hottentots Holland Mountains, both mericarps may develop. In *A. echinatus* and *A. monacanthus*, the fruit are rostrate (Figs. 7S, 9L, M) and brownish, whereas in *A. dregei* they are white and unbeaked (Fig. 5I). The fruit surface is spinescent in *A. echinatus* and *A. monacanthus*, while in *A. dregei* it has wart-like protuberances. Surface outgrowths on fruit are a common feature in the Saniculoideae (Drude, 1897–1898). In mature fruit, the abortive mericarp separates from the fully developed one in *A. echinatus* and *A. monacanthus*. In *A. dregei*, the developed carpel and the abortive carpel are slightly fused along their margins (Fig. 2A) so that they do not separate at maturity. A detailed study of mature fruit in transverse section (Fig. 2) revealed that the epidermis remains continuous between the two mericarps in *A. dregei* (Fig. 2A) but becomes discontinuous in *A. echinatus* (Fig. 2B) and *A. monacanthus* (Fig. 2C).

Much of the mesocarp becomes lignified in the mature fruit of *Arctopus dregei* (Fig. 2A) and *A. echinatus* (Fig. 2B) but less so in *A. monacanthus* (Fig. 2C). The innermost one or two cell layers of *A. monacanthus* (Fig. 2C) and one to five layers of *A. dregei* (Fig. 2A) and *A. echinatus* (Fig. 2B) are lignified. This was interpreted by Liu et al. (2003) as consisting of a single lignified endocarp layer with adjacent lignified mesodermal cells and therefore different from the multicellular lignified endocarp characteristic of Drude's Hydrocotyloideae (currently treated as Azorelloideae and Mackinlayoideae). In all three species of *Arctopus* (Fig. 2), the small rib oil ducts are always external to and associated with the numerous vascular bundles.

Phylogenetic Relationships

CLADISTIC ANALYSIS OF MORPHOLOGICAL DATA

In order to explore character evolution in *Arctopus*, a cladistic analysis was performed in which 15 characters were polarized (Table 2, Appendix 2). The African endemic saniculoid *Alepidea amatymbica* was selected as the outgroup because both *Alepidea* and *Arctopus* share similar kaurenoic acids (van Wyk et al., 1997), as well as numerous morphological characters (e.g., geophytes with simple leaves, toothed and occasionally inflexed leaf margins ending in setae, fused pseudanthial bracteoles and sessile female flowers). A single, shortest-length tree was produced with a tree

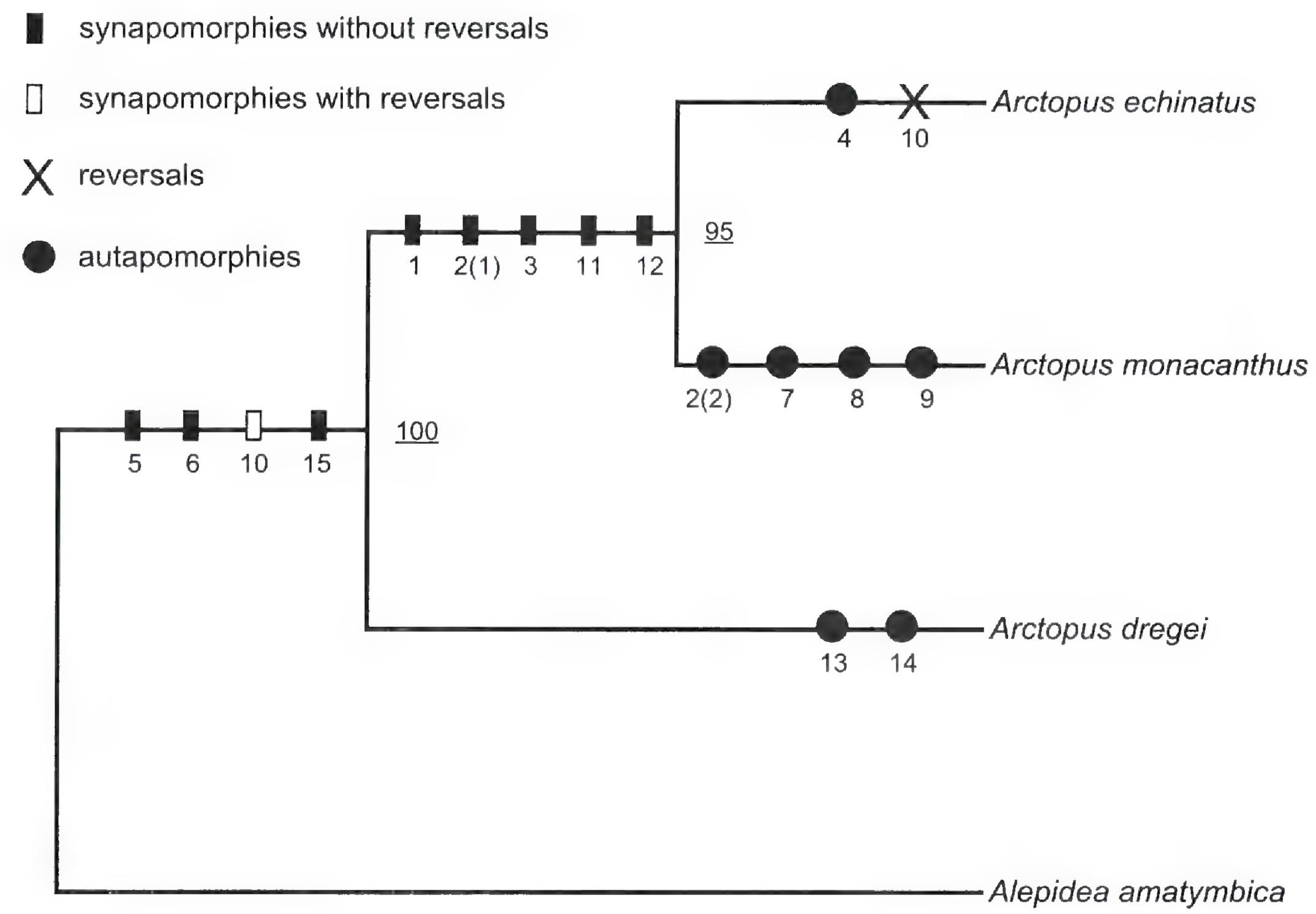

Figure 3. The single shortest-length tree (TL = 17 steps, CI = 0.94, RI = 0.83) obtained by a cladistic analysis of 15 morphological characters (Table 2) to explore possible relationships within the genus *Arctopus*. Characters and character states are numbered as in Appendix 2. Underlined numbers within the branch nodes are bootstrap percentages.

length (TL) of 17 steps, a consistency index (CI) of 0.94, and a retention index (RI) of 0.83 (Fig. 3). The monophyly of *Arctopus* is strongly supported (100 bootstrap percentage [BP]) by four rather convincing and unambiguous generic synapomorphies, namely the dioecious habit, the homochlamydeous perianth in female flowers, the pseudo-monocarpellate fruit, and the lignified endocarp. The pseudo-monocarpellate fruit, a generic synapomorphy, was scored as a reversal in *A. echinatus* because sporadic populations were found with mature fruit in which both mericarps had developed.

Arctopus echinatus and *A. monacanthus* are strongly supported as sister taxa (95 BP) by five synapomorphies. *Arctopus dregei* can be hypothesized to have retained more of the ancestral character states. There also appears to be a trend toward complete monospermy in *A. dregei* (perhaps an autapomorphy), with the carpels not separating from one another when mature and the epidermis remaining continuous between the two mericarps.

CLADISTIC ANALYSES OF ITS SEQUENCE DATA

The total number of characters from ITS-1, ITS-2, and the 5.8S gene was 687, of which 337 were variable (49.05%) and 232 (33.77%) potentially informative. Parsimony analysis produced six shortest-length trees (TL = 715 steps, CI = 0.72, RI = 0.73). The same overall topology was recovered in both the Bayesian (BI) and parsimony (MP) analyses, with the BI differing only in that *Sanicula* is more resolved, with a poorly supported clade (PP 0.75) consisting of *Sanicula arguta* Greene ex J. M. Coult. & Rose and *S. purpurea* H. St. John & Hosaka. A strict consensus of the six trees from MP is shown in Figure 4. It is interesting to note that ITS data produced the same overall topology as was found in a previous analysis (Calviño & Downie, 2007) based on chloroplast data (*trnQ-trnK* 5′: exon region). The tribe Steganotaenieae (represented here by *Steganotaenia araliacea* Hochst.) forms the earliest branching lineage within Saniculoideae (76 BP; PP 0.84) and *Alepidea* (represented here by *Alepidea amatymbica*) forms the earliest branching lineage within Saniculeae (50 BP; PP 0.98). In both analyses, the monophyly of *Arctopus* is strongly supported (100 BP; PP 1.0), but species relationships are unresolved.

Taxonomic Treatment

Arctopus L., Sp. Pl.: 1058. 1753. TYPE: *Arctopus echinatus* L.

Apradus Adans., Fam. Pl. 2: 102, 519. 1763, nom. illeg.

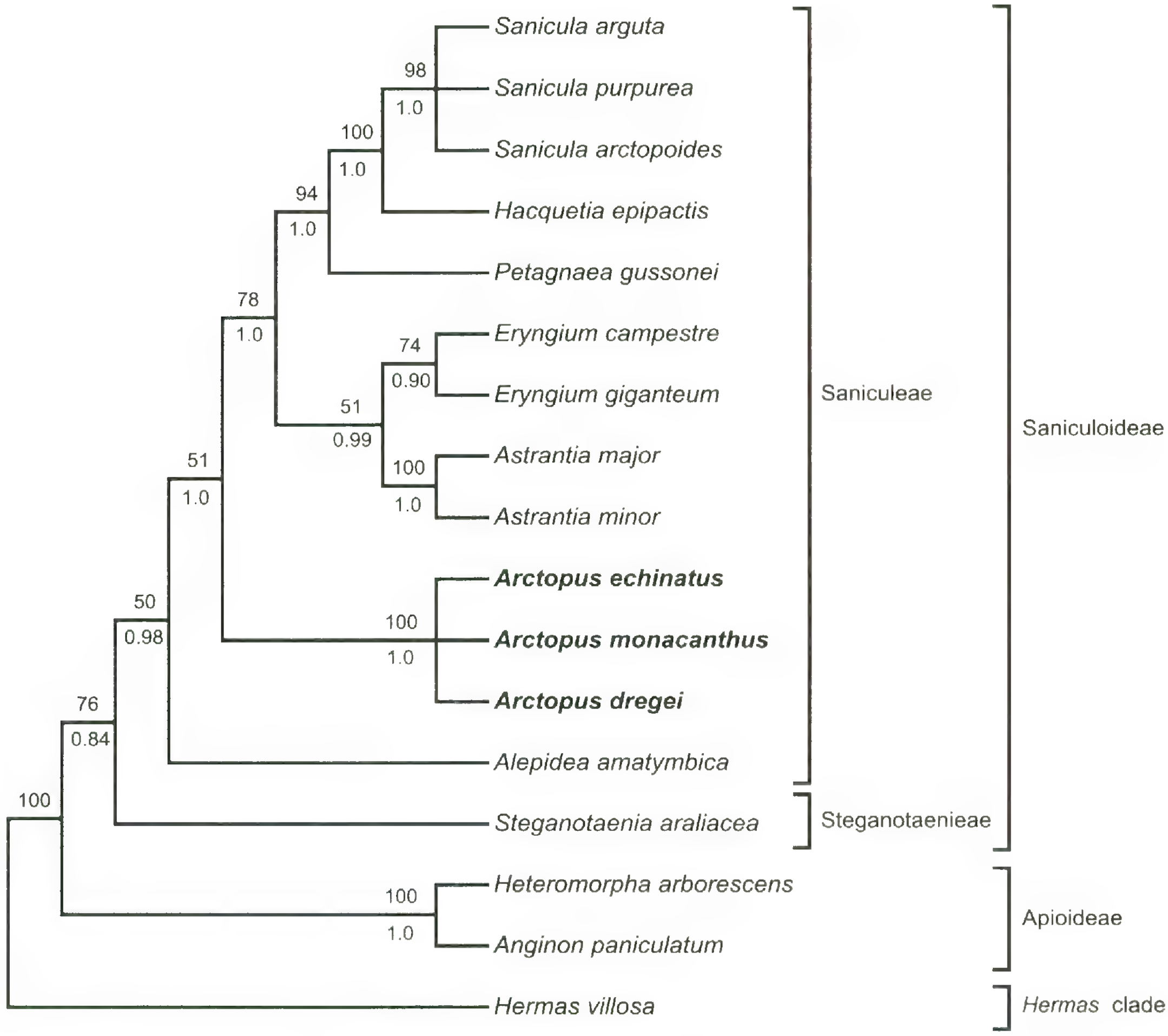

Figure 4. Strict consensus tree of six shortest-length 715-step trees derived from the parsimony analysis of the nrITS region (CI = 0.72, RI = 0.73). Numbers above the branches are bootstrap percentages above 50%, and those indicated below the branches are posterior probabilities obtained by Bayesian inference.

Dioecious, acaulescent, summer-deciduous geophytes; roots long, tuberous, ± 35 mm diam., resinous. Leaves simple, prostrate, spreading in a rosette with the inner smaller; ovate to semi-orbicular or rhomboidal, incised, 3-fid, each segment again 2- or 3-lobed, lobes dentate to crenate; margins toothed, setae spinose-flexuose; leaf base truncate to subtruncate or cuneate; glabrous on both sides; inflexed spine(s) usually present in leaf recesses between segments, often attached to an inflexed laminar tooth; venation palmate, 5- to 7-veined, sunken adaxially but prominently raised abaxially, secondary venation reticulate; petioles adaxially flattened, membrane-winged. Male inflorescence pedunculate, often with up to 5 lateral umbels; involucre spiny, of many linear or occasionally foliose bracts; rays 1 to 8; involucel spiny, of many ovate to lanceolate bracteoles; umbellule with more than 20 flowers. Male flowers pedicellate; sepals 5, linear-oblong, ± same size as petals; petals 5, white, oblong to oblanceolate, with inflexed tips, septum on inner face; stamens 5, twice as long as petals, tips inflexed; stylopodium flat, papillate; styles 2, short; ovary absent. Female inflorescence shortly pedunculate, often with up to 5 compound umbels; involucre as in male; rays 2 to 10; involucel accrescent, of 3 to 6 prominent, ovate or obovate, spiny bracteoles, forming a pseudanthium; bracteoles permanently fused to the fruit. Female flowers sessile, 3 to 5 per pseudanthium, tubular; sepals 5, triangular; petals 5 (the 10 perianth lobes on the female flowers are interpreted here as 5 sepals and 5 sepaloid petals; however, further ontogenetic studies are required), triangular, sepals and petals persistent in fruit; stamens absent; stylopodium cushion-shaped, raised above the style bases, papillate; styles 2, long, conical, longer than the sepals; ovary bilocular. Fruit usually rostrate, base adnate to the bracteoles, usually pseudo-monocarpellate due to abortion of inner mericarp.

Distribution. The genus is endemic to the Cape Floristic Region of South Africa. The rainfall is generally restricted to the winter months (Goldblatt & Manning, 2002) with only *Arctopus echinatus* extending into the almost year-round rainfall in the Eastern Cape Province. The three species share a sympatric distribution (Figs. 6, 8).

Phenology. The plants are dormant in summer and form new leaves in early winter (April). Flowering commences in June through September. Mature fruit are borne around November, at which time the leaves have begun to die back, followed by complete dormancy throughout the dry summer months.

Notes. The numerous unique and unusual characters in this genus make it immediately distinguishable from any other in the family. The species are dioecious geophytes with large, appressed, spiny leaves, female flowers that lack differentiation between sepals and petals, and pseudo-monocarpellate fruit that are surrounded by large, spiny, involucel bracteoles.

Adanson (1763–1764) superfluously described the genus *Apradus* from the same Plukenet (1691–1705: tab. 271, fig. 5) and Burman (1738: tab. 1) figures used earlier by Linnaeus (1753) when he described *Arctopus*. The name *Apradus* is, therefore, considered illegitimate here.

KEY TO THE SPECIES OF *ARCTOPUS*

1a. Female material (flowers sessile, leaves not required).
  2a. Involucel bracteoles flat or keeled.
    3a. Involucel bracteoles ovate, leathery, boat-shaped and keeled, with an acute apex . . . . . *A. echinatus*
    3b. Involucel bracteoles widely obovate, foliose and papery, flat, not keeled, with an obtuse apex . . . . . *A. monacanthus*
  2b. Involucel bracteoles strongly involute, not keeled . . . . . *A. dregei*
1b. Male material (flowers pedicellate) or leaves only.
  4a. Marginal teeth conspicuous so that lobes appear dentate; inflexed spines in leaf recesses typically present (except in a population around Milnerton, which has only sparsely spinescent leaves), involucel bracteoles ± as long as pedicels, 5–10 mm long.
    5a. Laminar tooth of inflexed spine(s) in leaf recesses absent or narrow, 0.5–1.0(–4.0) mm wide, setae almost always absent on tooth margins; petioles with cavities in transverse section . . . . . *A. echinatus*
    5b. Laminar tooth of inflexed spine(s) in leaf recesses usually broad, (2.0–)4.0–10.0 mm wide, setae usually present on tooth margins; petioles without cavities in transverse section . . . . . *A. monacanthus*
  4b. Marginal teeth inconspicuous so that lobes appear crenate; inflexed spines in leaf recesses usually absent, involucel bracteoles usually shorter than pedicels, 1–5 mm long . . . *A. dregei*

1. Arctopus dregei Sond. in Harv. & Sond., Fl. Cap. 2: 565. 1862. TYPE: South Africa. Western Cape Province: Agter de Paarl, sandy hills, s.d., *J. F. Drège 7649* (holotype, S!). Figure 5.

Leaves widely ovate or rhomboidal, blades 40–100 mm long and broad, not thorny; lobes crenate; margins inconspicuously toothed; setae flexuose, 1–5 mm; spine(s) in leaf recesses between segments usually absent, when present then inflexed, attached to an inflexed laminar tooth, inflexed part of lamina absent or narrow, less than 1 mm wide, setae absent, vertical spines 0(to 3), 5 mm; petioles 20–80 × 4–7 mm; wings broad, 1–4 mm wide. Male umbellule with pedicels longer than bracteoles, pedicels 1–10 mm; bracteoles lanceolate, spiny, 1–5 mm. Female inflorescence with rays 4–15 mm; pseudanthial bracteoles relatively small; 3 to 6; obovate, involute; ca. 8 × 4 mm at flowering stage, accrescent, becoming 9–20 × 5–10 mm at fruiting stage; apex obtuse, with a sharp spine, spine ca. 2 mm; margin with 1 or 2 inflexed, spinescent hairs; pseudanthial bracteoles becoming leathery at fruiting stage, reticulate venation not markedly prominent, becoming white when dried, not separating. Fruit not rostrate; 1-seeded; surface pustulate, white; developed mericarp ca. 8 × 5 mm; abortive mericarp small, not separating in mature fruit.

Distribution. Arctopus dregei was originally considered to be endemic to the Lowland Renosterveld of the Western Cape Province, from Paarl to Malmesbury, Yzerfontein, and Darling, and extending north to Hopefield. However, two recent collections of outlying populations around Vredenburg and Koekenaap in 2006 and 2007 have significantly extended the known geographical range for this species (Fig. 6) and shown for the first time that *A. dregei* is not restricted to Renosterveld, but may also occur in both Strandveld and Sandveld fynbos. It thrives in seasonally moist sandy soils and is often found in sparse vegetation dominated by Restionaceae (pers. obs.).

IUCN Red List category. Arctopus dregei is considered Vulnerable (VU B1ab[i,ii,iii,iv,v]) according to IUCN Red List criteria (IUCN, 2001). Lowland Renosterveld (the vegetation to which *A. dregei* is largely restricted) is considered to be one of the most threatened habitats in the Cape Floristic Region, with over 80% of the area completely transformed and currently only a few patches formally protected (Mucina & Rutherford, 2006). It is subject to

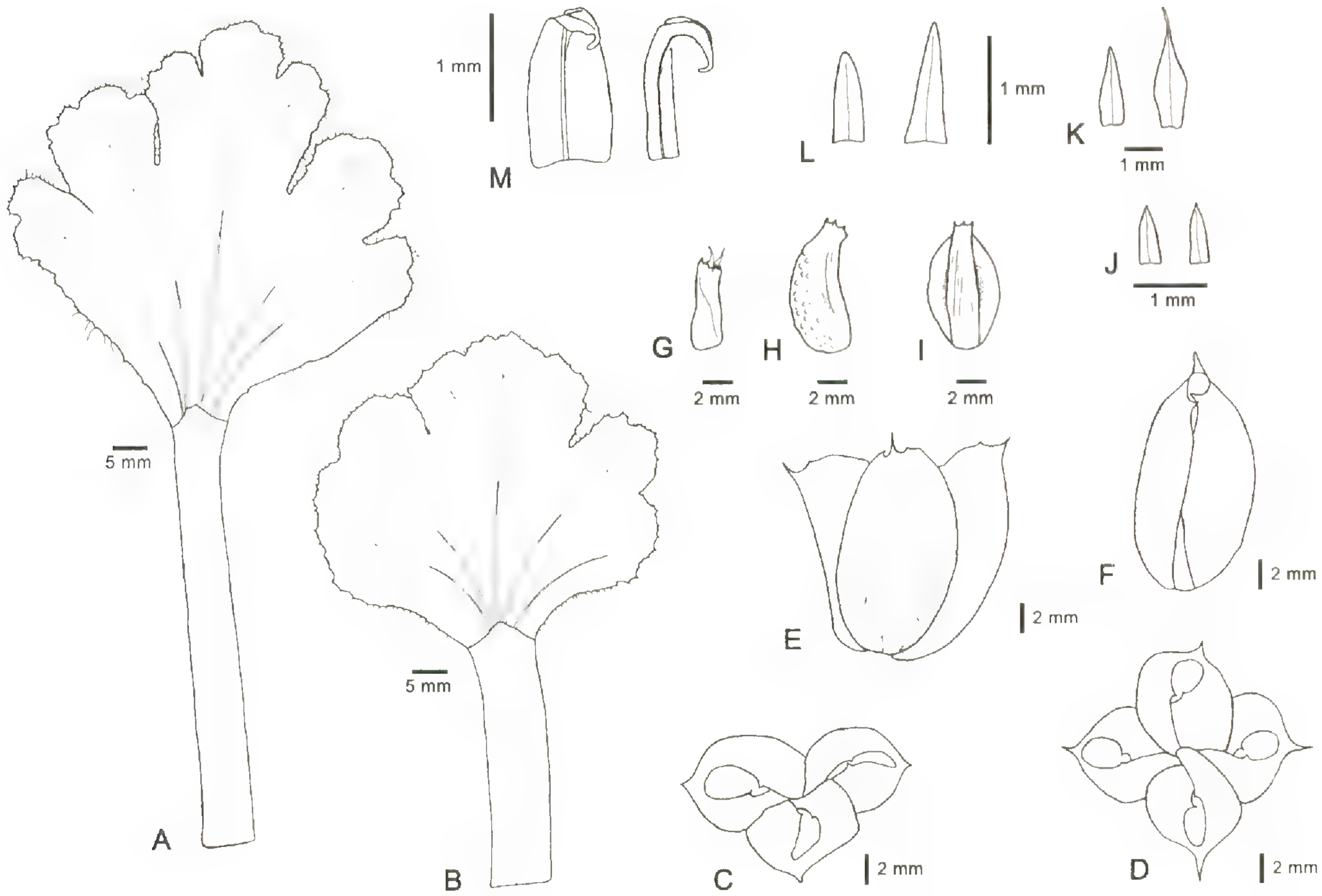

Figure 5. *Arctopus dregei*. —A, B. Leaves, adaxial view (A: *Magee & Boatwright 2*, JRAU; B: *Magee & Boatwright 5*, JRAU). C–J. Female flowers and fruits (*Magee & Boatwright 31*, JRAU). C F. Pseudanthium. —C, D. Top view. —E. Lateral view. —F. Single pseudanthium bracteole. —G. Female flower. —H. Fruit, lateral view. —I. Fruit, dorsal view. —J. Sepals and petals. K–M. Male flowers (*Boucher & Shepherd 4408*, NBG). —K. Bracteoles. —L. Sepals. —M. Petals.

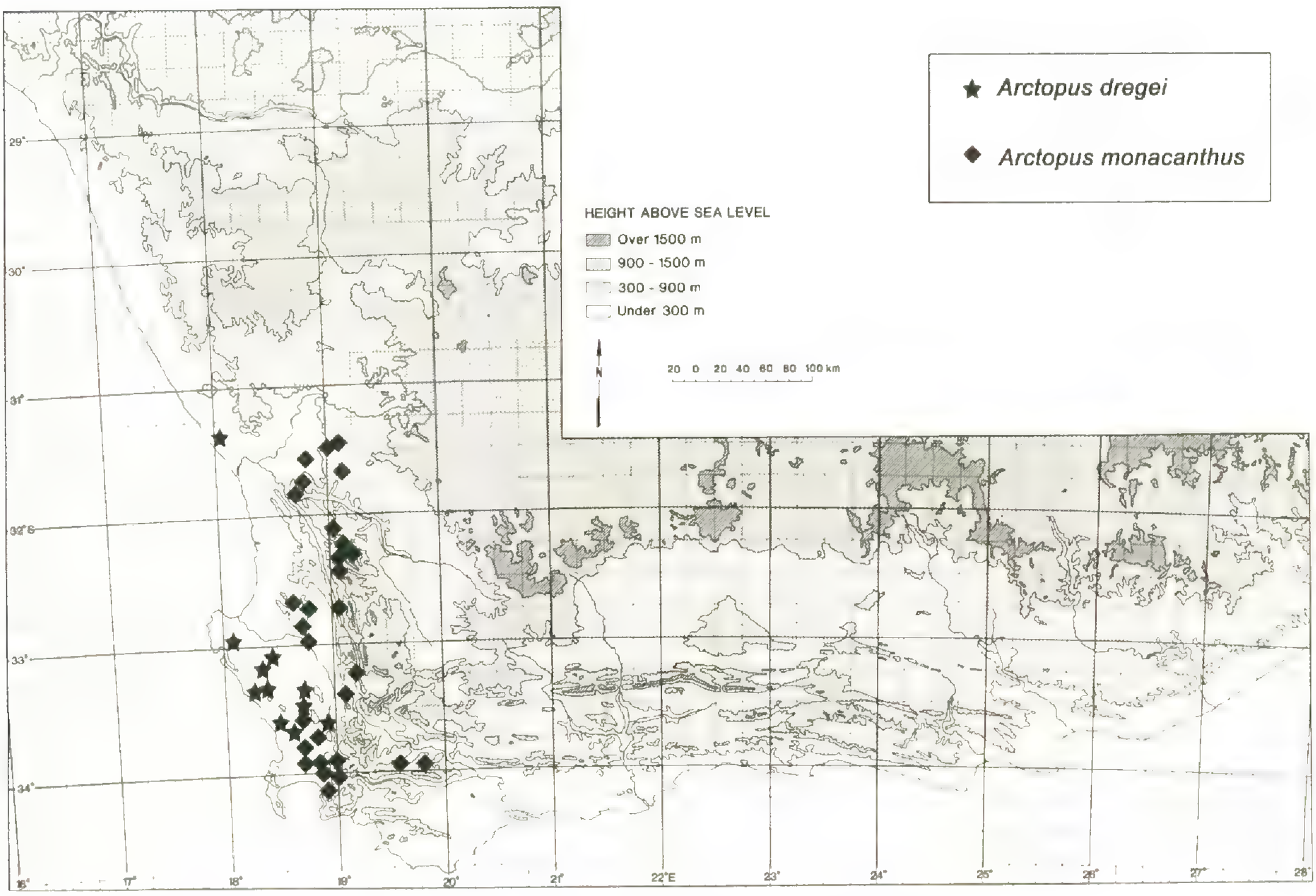

Figure 6. The known geographical distribution of *Arctopus dregei* and *A. monacanthus*.

extensive pressure in the form of agricultural expansion and seepage (drift sprays of herbicides and pesticides, as well as fertilizer runoff), and the spread of alien invasive species and urban encroachment and, as a result, is severely fragmented today. The species has also been recorded outside of Renosterveld; however, only two such populations are known. One of these is known to occur in Strandveld vegetation near Vredenburg and is threatened by proposed industrial expansion (*Helme 4181* [NBG], in schedula). Consequently, Helme and Raimondo (in prep.) have recommended that *A. dregei* be considered Vulnerable (VU) according to IUCN Red List criteria (IUCN, 2001) as it has a severely fragmented distribution and an extent of occurrence estimated to be 12,388 km^2.

Notes. *Arctopus dregei* (Fig. 5) is a distinctive species that can easily be distinguished by the crenate lobes of the leaves due to the inconspicuously toothed margins and the usually absent inflexed spines in the leaf recesses. The female bracteoles are involute. The male flowers have bracteoles that are shorter than the pedicels.

Additional specimens examined. SOUTH AFRICA. **Western Cape Province:** NW of Koekenaap, Farm Graafwater, *Goldblatt & Manning 12875* (NBG); 7 km SE of Vredenburg on Langeberg 187, N of R79, E of Namaqua Sands plant, *Helme 4181* (NBG); betw. Yzerfontein & Darling, *Magee & Boatwright 5* (JRAU); Hopefield, *Bolus 12699* (BOL, K); *Garside 1608* (K); Hopefield, Enkelvlei Farm, *Goldblatt & Manning 11414* (PRE); Yzerfontein, De la Rey Farm, *Louw 2529* (NBG); Malmesbury Allotment area, *Helme 2165* (NBG); Malmesbury, Klipfontein 598, *Helme 2574* (NBG); Malmesbury, *Magee & Boatwright 31* (JRAU); Malmesbury, Pella area, Burgers Post Farm, *Boucher & Shepherd 4408* (NBG); Malmesbury, Rustenburg Farm, *Helme 2719* (NBG); Malmesbury, Rondeberg Farm, *Helme 2763* (NBG); *Magee & Boatwright 1, 2, 3, 4* (JRAU); Paarl, *Acocks 2440* (K, PRE). Precise locality unknown: *Drège s.n.* (BM).

2. Arctopus echinatus L., Sp. Pl.: 1058. 1753. TYPE: South Africa. Western Cape Province: Cape Town, Signal Hill near Kramat, 16 July 2003, *J. Manning & G. Reeves 2845* (proposed conserved type, designated by van Wyk et al., 2006: 541, NBG!). Figure 7.

Leaves widely to very widely ovate, semi-orbicular or rhomboidal, blades 20–80 mm long and broad, thorny (sparsely thorny in the Milnerton population); lobes dentate to doubly dentate, rarely crenate (Milnerton population); margins conspicuously toothed; setae spinose, rarely flexuose, 3–10 mm; spine(s) in leaf recesses between segments inflexed, occasionally attached to an inflexed laminar tooth; inflexed part of lamina absent or narrow, 0.5–1.0 (–4.0) mm wide, setae absent, vertical spines 1 to 3(4), stout, 6–15 mm; petioles 20–200 × 5–7 mm; wings narrow, ca. 1 mm wide. Male umbellules with pedicels ± as long as the bracteoles; bracteoles ovate to lanceolate, spiny, 5–10 mm. Female inflorescence with rays 10–40 mm; pseudanthial bracteoles relatively small, 4 or 5, ovate, boat-shaped and keeled; ca. 6 × 3 mm at flowering stage, accrescent, becoming 10–18 × 5–8 mm at fruiting stage; apex acute, with a sharp spine, spine 2–3 mm; margin with 1 or 2 inflexed and overlapping spines; pseudanthial bracteoles becoming leathery and firm at fruiting stage, prominently thickened along periphery and midrib, becoming brown when dried, not separating. Fruit rostrate; usually 1-seeded, rarely 2-seeded; surface usually spiny, brown; developed mericarp ca. 10 × 5 mm; abortive mericarp small, separating in mature fruit.

Distribution. *Arctopus echinatus* is the most widespread species of the genus, occurring throughout the Western Cape Province and extending both north to Nieuwoudtville in the Northern Cape Province and east along the coast to Port Alfred in the Eastern Cape Province (Fig. 8). Plants are usually found in seasonally moist sandy soils (pers. obs.).

IUCN Red List category. *Arctopus echinatus* is considered Least Concern (LC) according to IUCN Red List criteria (IUCN, 2001).

Notes. *Arctopus echinatus* (Fig. 7) can be distinguished from *A. dregei* by the dentate lobes on the leaves, due to the conspicuously toothed margins, and the presence of inflexed spines in the leaf recesses. More notably, the female bracteoles are ovate, boat-shaped and keeled, with an acute apex. The male flowers have bracteoles that are equal in length to the pedicels. This species differs from *A. monacanthus* by the narrow or absent inflexed laminar tooth of the spine in the leaf recesses, which usually lacks setae. The leaf anatomy also differs from that of *A. monacanthus* in that the petiole in transverse section has cavities and the adaxial vascular bundle of the midrib is either simple or dorsiventrally split.

Burtt (in Jarvis et al., 1993: 20) designated the Burman plate (tab. 1 in J. Burman, Rar. Afric. Pl. 1738) as lectotype of *Arctopus echinatus*. Unfortunately, the plant illustrated in this plate was not identifiable as *A. echinatus* of current usage, but rather as *A. monacanthus*. Van Wyk et al. (2006) therefore proposed the conservation of *A. echinatus* with a conserved type in order to maintain the widespread usage of this name. This proposal has been recommended by the Nomenclature Committee (Brummit, 2007: 1293).

Additional specimens examined. SOUTH AFRICA. **Eastern Cape Province:** Uitenhage, *Drège s.n.* (K), *Zeyher s.n.*

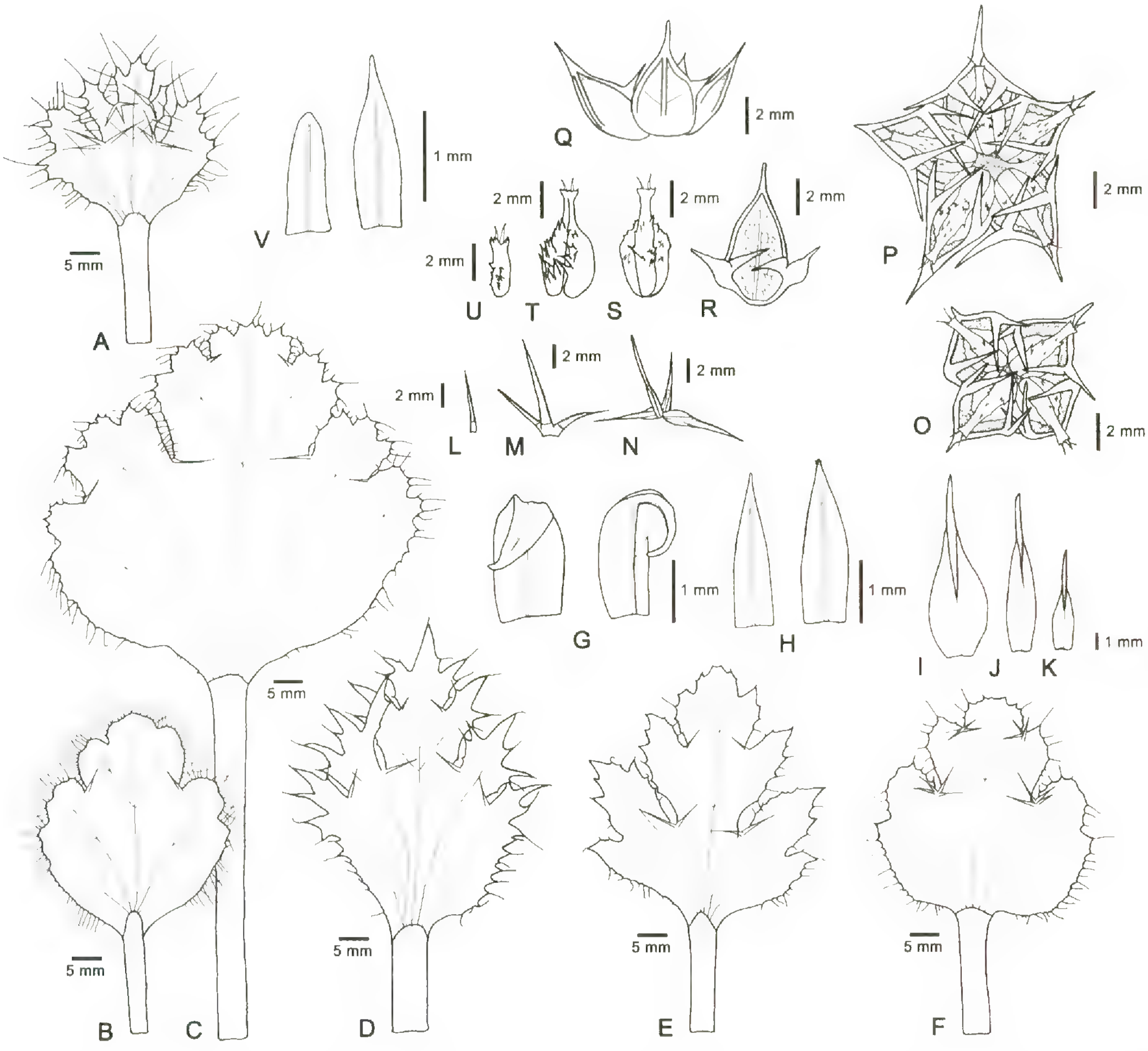

Figure 7. *Arctopus echinatus*. —A–F. Leaves, adaxial view (A: *Esterhuysen 12231*, BOL; B: *Adamson 2517*, BOL; C: *Magee & Boatwright 6*, JRAU; D: *Magee & Boatwright 15*, JRAU; E: *Theron 1764*, PRE; F: *Tilney 257*, JRAU). G–K. Male flowers (G, H: *Van Wyk & Viljoen 3715*, JRAU; I: *Rycroft 1939*, NBG; J: *Bond 1149*, NBG; K: *Adamson 2865*, PRE). —G. Petals. —H. Sepals. —I–K. Bracteoles. —L–N. Inflexed lamina spines (L: *Taylor 5099*, PRE; M: *Magee & Boatwright 15*, JRAU; N: *Brink 767*, PRE). O–V. Female flowers and fruits (*Magee & Boatwright 6*, JRAU). O–R. Pseudanthium. —O, P. Top view. —Q. Lateral view. —R. Single pseudanthium bracteole. —S. Fruit, dorsal view. —T. Fruit, lateral view. —U. Flower. —V. Sepals and petals.

(BM); Paterson, *Retief 471* (PRE); Port Elizabeth, *Bolus 2246* (BOL, K), *Borle 28* (PRE), *Patterson 25755* (PRE); Port Elizabeth, Victoria Park Lands, *Long 618* (K, PRE); Botha's Hill, *Rodgers 4438* (BOL); Alexandria, *Galpin 10762* (K, PRE), *Watt 1445* (PRE); Alexandria, Bushmans River Mouth, *Archibald 3634* (K, PRE), *Marais 409* (K, PRE); Alexandria, Bokness village, *Brink 767* (PRE); Kasouga, *Tilney 257* (JRAU); Kowie, *Britten 2135* (PRE), *Tyson 19229* (PRE); Bathurst, *Henrici 12* (PRE); Port Alfred, *Hutton s.n.* (PRE). **Northern Cape Province:** Van Rhyns Pass top, *Bond 1149* (NBG), *Taylor 2908* (NBG), *Van der Schijff 7179* (PRE); Nieuwoudtville, *Van Wyk 4128a* (JRAU); Aarendskraal, *Van Wyk s.n.* (JRAU); Oorlogskloof, *Van Wyk & Viljoen 3715* (JRAU); Oorlogskloof farm, Tuinlaagte, *Magee et al. 93* (JRAU). **Western Cape Province:** Cederberg, betw. Middelberg hut & Crystal Pool, *Barnes 19284* (BOL); Cederberg, Scorpionspoort, *Esterhuysen 12231* (BOL); Cedarberg, *Thode A2003* (PRE); 11 km from Op-Die-Berg to Cedarberg, *Stirton 9162* (PRE); Rondegat River valley, *Le Maitre 337* (PRE); Sneeuberg, Cedarberg Forest Reserve, *Taylor 5099* (PRE); Langebaan Lagoon, *Axelson 443* (NBG); Darling Flora Reserve, *Rycroft 1939* (NBG); betw. Yzerfontein & Darling, *Magee & Boatwright 6* (JRAU); Sea Point, *MacOwan 1624* (BM, S), *Phillips s.n.* (BM); Clifton, *Acocks 4566* (S), *Barker 1528* (NBG); Rondebosch Common, *Barker 1529* (NBG); Claremont, *Salter 9231* (BM); Wynberg Hill, *Salter 9224* (BM); Wynberg, *Gamble 22123* (K); Table Mtn., *Fries 3133* (S), *Peter 50305* (K); Signal Hill, *Blake s.n.* (NBG); *Marloth 5562* (PRE); Lions Rump, *Thode A105* (NBG, PRE), *Worsdell s.n.* (K); Milnerton, *Adamson 2517* (BOL), *2865* (BOL, PRE), *Hanson 2517* (BOL), *Salter 8199* (BOL); Killarney, *Lussem 17* (NBG); Porterville, Grootwinterhoek Mtn., *Barker 262* (PRE); Stellenbosch, *Garside 1506* (K), *Kerfoot 5842* (PRE) [atypical, possible hybrid with *Arctopus monacanthus*], *Moss 4046* (BM); Gydo Pass, *Johnson 505* (NBG); Ceres, *C. M. Van Wyk 3319* (PRE); Ceres, Skoongesig, *Hanekom 662 & 663* (PRE); Worcester Veld Reserve, *Olivier 61* (PRE); Worcester, *Van Breda 71* (PRE); Worcester, Langerug koppie, *Walters 1991 & 1992* (NBG); Du Tooits Kloof pass, *Winter et al. 170* (JRAU); Botrivier

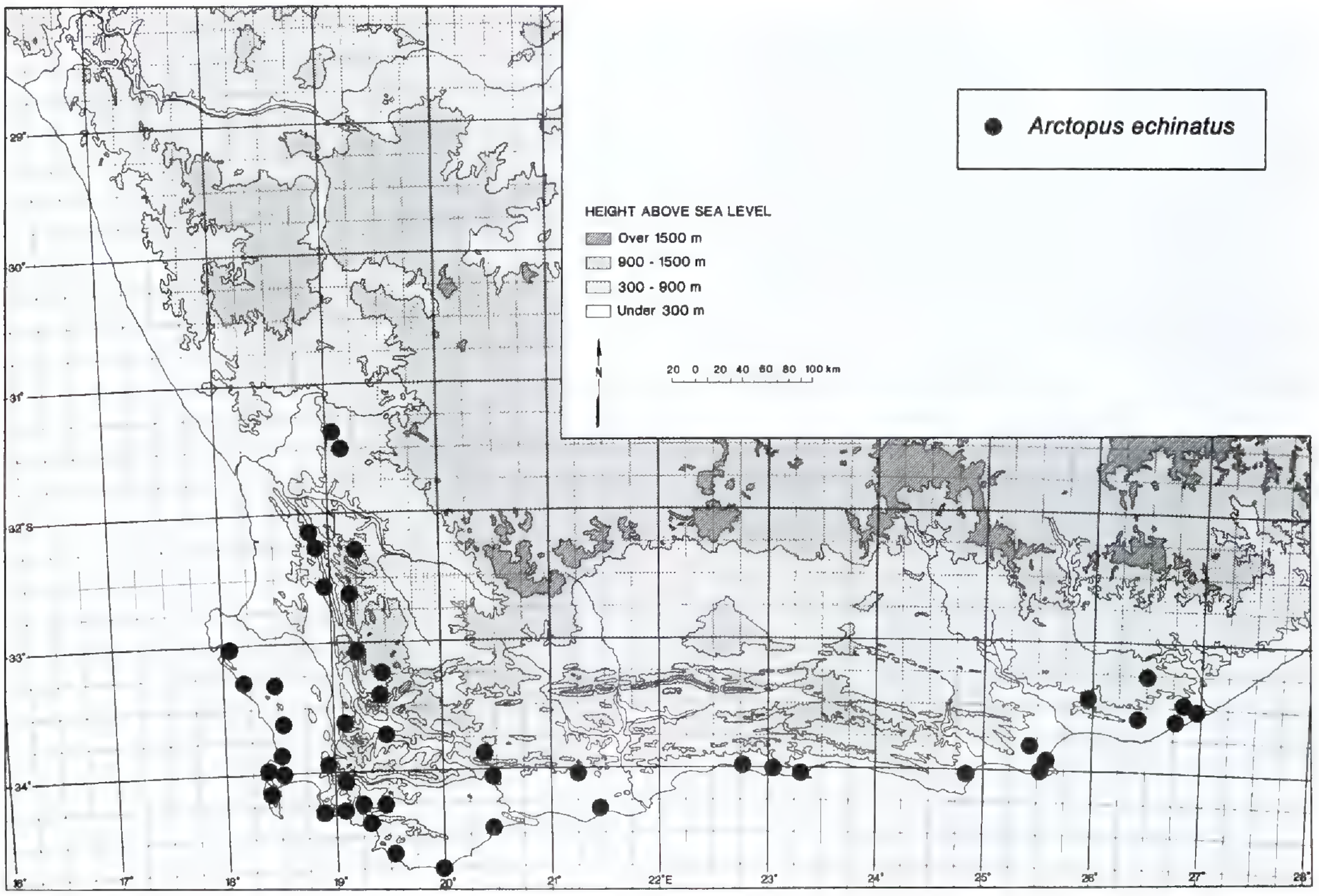

Figure 8. The known geographical distribution of *Arctopus echinatus*.

vlei, *Loubser s.n.* (NBG); Clock Peaks, *Wurts 148* (NBG); Constantianek, *Young 26521* (PRE); Simon's Town, *Alice & Godman 67* (BM); Betty's Bay, Dawidskraal, *Boucher 1379* (NBG); Kleinmond, *Burman 1171* (BOL), *De Vos 607* (NBG); Palmiet, *De Vos 719* (NBG); Hermanus, Fernkloof Nature Reserve, *Orchard 558* (K, NBG, PRE), *Magee & Boatwright 15* (NBG); Hermanus, Westcliff, *Williams 303* (K); Pearly Beach, Kleinhagelkraal, *C. M. Van Wyk 616* (NBG, PRE); Zoetendalsvlei, *Smith 3098* (PRE); De Hoop flats near Die Mond, *Burgers 2576* (NBG); De Hoop, *Van der Merwe 800* (PRE); Soetmelksfontein, *Muir 5204* (PRE); Riversdale, Still Bay, *Bohnen 7540* (NBG, PRE); Riversdale, *Muir 24400* (BOL); Knysna, *Theron 1764* (PRE); Kranshoek lookout point, near Harkerville, *Boatwright, Magee & Van Wyk 4* (JRAU); Humansdorp, Blouleliesbos, *Geldenhuys 99* (PRE). Precise locality unknown: *Sieber 141* (S).

3. Arctopus monacanthus Carmich. ex Sond., Fl. Cap. 2: 565. 1862. TYPE: South Africa. Cape Province: s.d., *Captain Carmichael s.n.* (holotype, S!). Figure 9.

Leaves widely to very widely ovate, semi-orbicular or rhomboidal, blades 25–120 mm long and broad, thorny; lobes dentate to doubly dentate; margins conspicuously toothed; setae spinose, 3–7 mm; spine(s) in leaf recesses between segments inflexed, attached to an inflexed laminar tooth; inflexed part of lamina usually broad, (2.0–)4.0–10.0 mm wide, setae present, vertical spines 1 to 5(6), stout, 2–20 mm; petioles 20–100 × 3–7 mm; wings narrow, ca. 1 mm wide. Male umbellules with pedicels ± as long as bracteoles; bracteoles lanceolate or occasionally ovate, spiny, 5–10 mm. Female inflorescence with rays 10–50 mm; pseudanthial bracteoles large, 4 or 5, widely obovate, foliose, ca. 10 × 3 mm at flowering stage, massively accrescent, becoming 20–40 × 15–50 mm at fruiting stage; apex obtuse, with a sharp spine, spine ca. 4 mm; margin entire, rarely with 1 to 3 spines; pseudanthial bracteoles becoming brittle and papery at fruiting stage, reticulate venation prominent, becoming brown when dried, breaking away (with the ripe fruit attached). Fruit markedly rostrate; 1-seeded; surface occasionally spiny, brown; developed mericarp ca. 15 × 5 mm; abortive mericarp small, separating in mature fruit.

Distribution. *Arctopus monacanthus* has an extreme western distribution occurring from Somerset West in the Western Cape Province, north toward Nieuwoudtville in the Northern Cape Province (Fig. 6). It is an inland species occurring in seasonally moist clay soils or, less often, on sand (pers. obs.).

IUCN Red List category. *Arctopus monacanthus* is considered Least Concern (LC) according to IUCN Red List criteria (IUCN, 2001).

Notes. *Arctopus monacanthus* (Fig. 9) is similar to *A. echinatus*, but differs in having a broad inflexed laminar tooth in each of the leaf recesses with setae usually present on the margins of these teeth. The

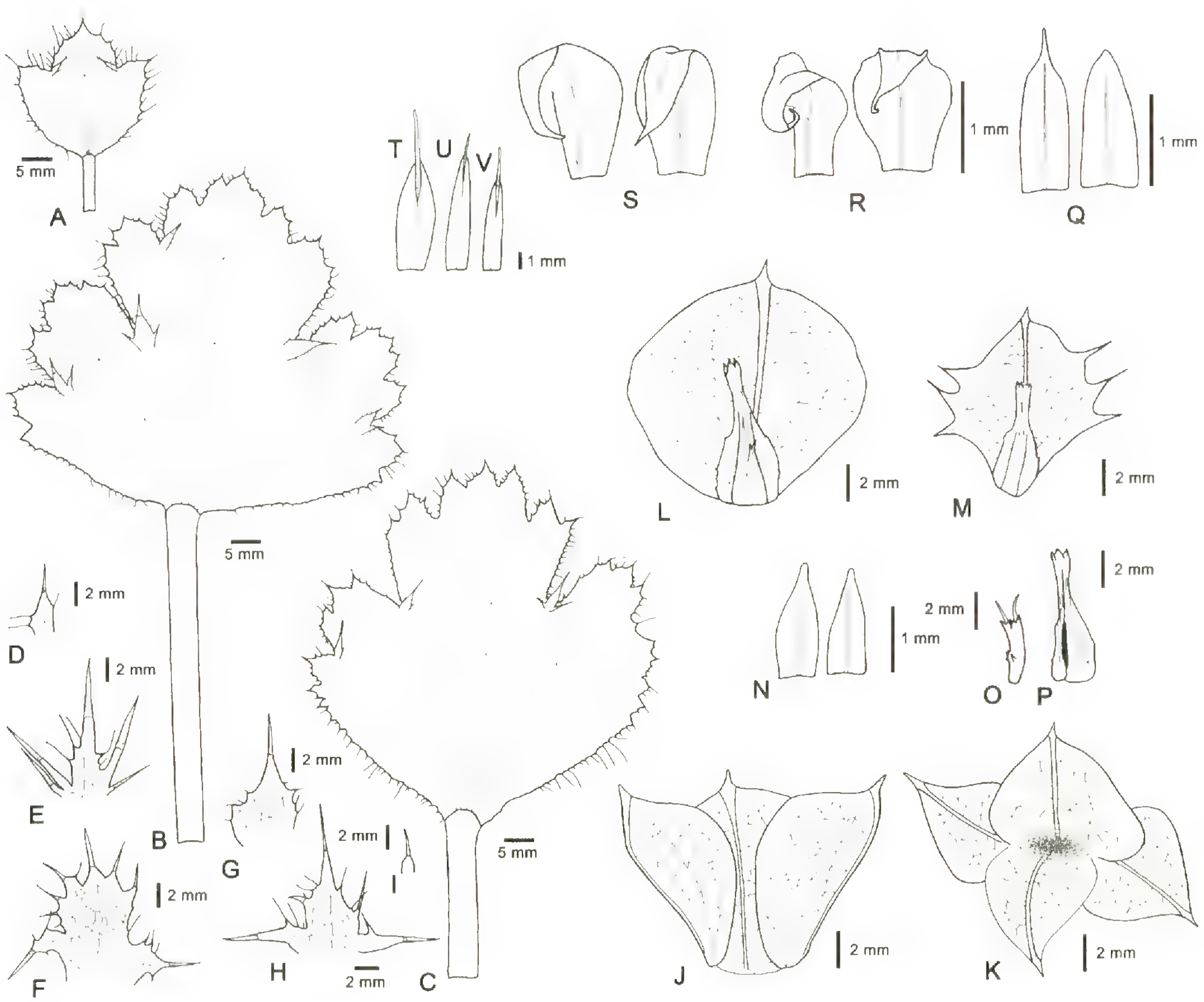

Figure 9. *Arctopus monacanthus*. —A–C. Leaves, adaxial view (A: *Acocks 17286*, PRE; B: *Mauve & Hugo 49*, PRE; C: *Van Wyk 4141a*, JRAU). —D–I. Inflexed lamina spines (D: *Van Wyk 4128b*, JRAU; E: *Barker 9882*, NBG; F: *Loubser 3348*, NBG; G: *Van Zyl 3140a*, NBG; H: *Runnalls 511*, NBG; I: *Van Wyk 3522*, JRAU). J–P. Female flowers and fruits (J, M, P: *Van Wyk 4161a*, JRAU; N, O: *Magee et al. 96*, JRAU). J–M. Pseudanthium. —J. Lateral view. —K. Top view. —L, M. Single pseudanthium bracteoles, with fruit in dorsal view. —N. Sepals. —O. Flower. —P. Fruit, lateral view. Q–V. Male flowers (Q, S, T: *Boucher 4389*, NBG; U: *Runnalls 512*, NBG; R, V: *Parker 3518*, PRE). —Q. Sepals. —R, S. Petals. —T–V. Bracteoles.

female bracteoles are widely obovate and foliose with an obtuse apex. In transverse section, the petiole is without cavities and the adaxial vascular bundle of the midrib is collaterally split.

Additional specimens examined. SOUTH AFRICA. **Northern Cape Province:** Nieuwoudtville, *Van Wyk 4128b* (JRAU); Nieuwoudtville, Oorlogskloof Nature Reserve, *Pretorius 181504* (NBG); Oorlogskloof farm, Tuinlaagte, *Magee et al. 96* (JRAU); Heerenlogement, *Barker 9882* (NBG); Gifberg, *Van Wyk 3522* & *4161a* (JRAU); Lokenburg, *Acocks 17286* (PRE). **Western Cape Province:** Piqueniers Kloof, *Oskolski et al. 35-06* (JRAU), *Schlechter 10756* (BM, BOL, K); Piquetberg, De Hoek, *Steyn 540* (NBG); Piquetberg, *Laers 19629* (BOL), *Stephens & Glover 8710* (BOL, K), *Van Wyk 3594* (JRAU); Pakhuis Pass top, *Wisura 2982* (NBG), *Magee & Boatwright 34* (JRAU); Clanwilliam, 20 km along rd. to Algeria, *Scott 393* (BOL); Wupperthal, *MacOwan 3236* (K); Cedarberg, Middelberg West, *Viviers 514* (PRE); Middleberg pass, *Van Wyk s.n.* (JRAU); Oliphants River Valley, opposite Warm Bath, *Stephens 7246* (K); Durbanville, Klipheuwel Farm, *Van Zyl 3140a* (NBG); Malmesbury, N side of Hercules Pillar, *Adamson 3168* (BM, BOL); Dassenberg, summit of Kanonkop, *Boucher 4389* (NBG); Tygerberg Nature Reserve, *Loubser 3348* (NBG); Langverwacht above Kuils River, *Olivier 4686* (NBG); Bottelaryberg, *Acock 2511* (S); Stellenbosch, *Bos 328* (PRE), *Gillett 583* (NBG); Stellenbosch, Vlottenberg station, *Stegmann 10566* (BOL, PRE); Straatkerk, 3 km W of Tulbagh, *Mauve & Hugo 49* (PRE); Elandskloof pass, *Van Wyk 4141a* (JRAU); Jonaskop, *Van Wyk s.n.* (JRAU); McGregor rd., *Van Wyk, Winter & Tilney s.n.* (JRAU); Somerset West, *Boucher & Mauve 4948* (PRE), *Parker 3518* (BOL, K, NBG, PRE); Somerset West, Helderberg Nature Reserve, *Runnalls 511* & *512* (NBG); Somerset in Hottentots Holland Mtn., *Ecklon & Zeyher s.n.* (S). Precise locality unknown: Malmesbury–Clanwilliam rd., *Alice & Godman 514* (BM).

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Appendix 1. List of the voucher specimens of taxa used in the molecular analysis, with GenBank accession numbers.

New Sequence Data

Azorelloideae: *Hermas villosa* Thunb., *A. R. Magee & J. S. Boatwright 24* (JRAU), AM748815. **Saniculoideae:** *Hepidea amatymbica* Eckl. & Zeyh., *A. R. Magee & J. S. Boatwright 45* (JRAU), AM158945; *Arctopus dregei* Sond., *A. R. Magee & J. S. Boatwright 2* (JRAU), AM158942; *Arctopus echinatus* L., *A. R. Magee & J. S. Boatwright 6* (JRAU), AM158943; *Arctopus monacanthus* Carmich. ex Sond., *B.-E. van Wyk 3522* (JRAU), AM158944; *Steganotaenia araliacea* Hochst., *O. Maurin 566* (JRAU), AM748814.

Sequence Data from GenBank

Apioideae: *Anginon paniculatum* (Thunb.) B. L. Burtt, AF467922 (Neves & Watson, 2004); *Heteromorpha arborescens* Cham. & Schltdl., U27578 (Downie & Katz-Downie, 1996). **Saniculoideae:** *Astrantia major* L., AF077876 (Downie & Katz-Downie, 1996); *Astrantia minor* L., AF337183, AF337191 (Valiejo-Roman et al., 2002); *Eryngium campestre* L., AF077887 (Valiejo-Roman et al., 1998); *Eryngium giganteum* Bieberd., AF337182, AF337190 (Valiejo-Roman et al., 2002); *Hacquetia epipactis* DC., AF077892 (Valiejo-Roman et al., 1998); *Petagnaea gussonei* (Spreng.) Rauschert, AM403487, AM403486 (De Castro, unpublished); *Sanicula arctopoides* Hook. & Arn., AF031974 (Vargas et al., 1998); *Sanicula arguta* Greene ex J. M. Coult. & Rose, AF031975 (Vargas et al., 1998); *Sanicula purpurea* H. St. John & Hosaka, AF031971 (Vargas et al., 1998).

Appendix 2. Morphological characters and character states used for cladistic analysis of *Arctopus*.

1. **Setae on leaf margins:** 0 = flexuose; 1 = spinose.
2. **Inflexed laminar teeth in the recesses between the leaf segments:** 0 = absent; 1 = present, narrow (< 2(–4) mm); 2 = present, broad (> 4 mm).
3. **Collenchyma in petiole wings:** 0 = absent; 1 = present.
4. **Petiole cavities:** 0 = absent; 1 = present.
5. **Sexual system:** 0 = monoecious; 1 = dioecious.
6. **Sepals and petals of female flowers:** 0 = distinct sepals and petals; 1 = homochlamydeous perianth.
7. **Size of pseudanthium bracteoles:** 0 = small; 1 = large.
8. **Shape of pseudanthium apex:** 0 = acute; 1 = obtuse.
9. **Texture of pseudanthium:** 0 = leathery; 1 = papery.
10. **Fruits:** 0 = bicarpellate; 1 = pseudo-monocarpellate.
11. **Fruit shape:** 0 = not rostrate; 1 = rostrate.
12. **Fruit surface:** 0 = protuberant; 1 = spinescent.
13. **Mature fruits:** 0 = separating; 1 = not separating.
14. **Fruit epidermis between mericarps of mature fruits:** 0 = discontinuous; 1 = continuous.
15. **Endocarp:** 0 = parenchymatous; 1 = lignified.

A NATURAL HYBRID BETWEEN *LIGULARIA PARADOXA* AND *L. DUCIFORMIS* (ASTERACEAE, SENECIONEAE) FROM YUNNAN, CHINA[1]

Yuezhi Pan,[2] Suhua Shi,[3] Xun Gong,[2,3] and Chiaki Kuroda[4]*

ABSTRACT

A putative natural hybrid in *Ligularia* Cass., which is morphologically intermediate between *L. paradoxa* Hand.-Mazz. and *L. duciformis* (C. Winkl.) Hand.-Mazz., was found on Mt. Maoniu in northwestern Yunnan, China. We employed analyses of morphology, seed vigor, somatic chromosome numbers, meiotic behavior of chromosomes, inter-simple sequence repeat (ISSR) markers, and *trnL-F* sequencing to test the hypothesis that the unidentified taxon is a natural interspecific hybrid between *L. paradoxa* and *L. duciformis*. Examination of morphological characters demonstrated the intermediacy of the putative natural hybrid between *L. paradoxa* and *L. duciformis*. Seed germination of the putative natural hybrid was absent, and no embryo was found in seeds of the putative natural hybrid. The meiotic behavior of the putative natural hybrid was abnormal. Among 10 ISSR primers that we tested in all three taxa, four primers produced different ISSR markers between the putative parents. The putative natural hybrid shared all ISSR markers of the putative parents. However, no taxon-specific band existed in the samples of the putative natural hybrid. The data from ISSR markers are concordant with morphology in implicating *L. paradoxa* and *L. duciformis* as parents of the putative natural hybrid. *Ligularia paradoxa* was determined as the female parent of this natural hybrid by nucleotide sequence from the chloroplast *trnL-F* region. Therefore, this taxon is confirmed to be a natural hybrid between *L. paradoxa* and *L. duciformis* and is described herein.

Key words: Asteraceae, IUCN Red List, *Ligularia*, natural hybrid, Senecioneae.

Hybridization is common in many groups of flowering plants (Stebbins, 1959; Grant, 1981; Ellstrand et al., 1996) and is increasingly recognized as an important source of evolutionary novelty (Arnold, 1997; Rieseberg, 1997). Hybrids can arise in any situation where genetic divergence is not closely accompanied by development of a reproductive barrier, thus creating the potential for gene flow between entities that are taxonomically recognizable and morphologically distinct. The consequences of hybridization may include introgression affecting one or both taxa, formation of hybrid (especially alloploid) species, and development of reticulate patterns of evolution within a group (Arnold, 1997).

Although hybridization in combination with polyploidy has long been accepted as a mechanism of speciation in plants (Stebbins, 1971; Levin, 1983), the increasing use of molecular approaches has facilitated understanding of this process and revealed many new examples (Barton, 1989; Wendel, 1989; Soltis et al., 1991; Harris & Ingram, 1992; Soltis & Soltis, 1993; Brochmann et al., 1996; Sun, 1996; Sang et al., 1997; Padgett et al., 1998; Wolfe et al., 1998; Aparicio et al., 2000; Barton et al., 2001; Choi et al., 2001; Allen, 2002). Molecular data can yield many insights into the nature and parentage of hybrid species and, as further cases are investigated, into the circumstances that favor allopolyploid speciation. They are most commonly observed in outcrossing, perennial taxa, typically those with some mode of vegetative propagation.

The genus *Ligularia* Cass. (Asteraceae, Senecioneae) consists of about 130 species mainly distributed in eastern Asia, 112 species of which are known in China (Liu et al., 1994). Sixty-seven species were recognized in the Hengduan Mountains in southwestern China; of them, 61 species are endemic to this area (Liu, 1989). The Hengduan Mountains have been

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considered a main center of evolution and diversification of *Ligularia* (Liu et al., 1994). *Ligularia* is a typical alpine genus and a key taxon to studies on the origin and development of the Sino-Himalayan alpine floristic elements of flora. Although *Ligularia* has been taxonomically studied by Handel-Mazzetti (1938), Hu (1967), and Liu (1985, 1989), no molecular phylogenetic studies have been reported yet. The authors are doing a comprehensive study on the phylogeny of *Ligularia* using various methods to investigate its morphology, cytology, and molecular biology.

During field collection trips to the Hengduan Mountains in Yunnan, we found a mixed population of three taxa, namely *Ligularia paradoxa* Hand.-Mazz., *L. duciformis* (C. Winkl.) Hand.-Mazz., and a putative natural hybrid, occurring on Mt. Maoniu. This mixed population grows along a stream in the forest at an altitude of 4200 m. The putative nothospecies was considered to be a natural hybrid between *L. paradoxa* and *L. duciformis* based on its intermediate morphological appearance and the partial overlap of the flowering period of two putative parents. Given this anthesal overlap, cross-pollination between two putative parents could occur naturally under sympatric conditions.

The aim of the present study is to clarify the taxonomic status of the putative natural hybrid of *Ligularia duciformis* and *L. paradoxa* found on Mt. Maoniu, and to determine systematic relationships among these three taxa using various taxonomic approaches, including comparison of external morphology, seed vigor, examination of somatic chromosome number and meiotic behavior, inter-simple sequence repeat (ISSR) markers, and *trnL-F* sequencing.

Materials and Methods

Morphological observation was carried out in the field during the 2001 and 2002 flowering seasons. Leaf form and pappus color were selected among morphological characters as diagnostic characters. For studies on seed vigor, achenes of the three taxa were collected in October 2001. Embryos of all three taxa were observed under a light microscope. Achene weights were measured based on 1000 achenes, and the germination rates of achenes, sown in soil, were counted.

Somatic chromosome numbers were examined by the conventional aceto-orcein squash method using root tips. Root tips were collected from five living individuals per taxon in the field and then were pretreated with 0.1% colchicine solution for 3 hr. After fixation with a 3:1 mixture of 95% ethanol and glacial acetic acid at 4°C for 15 min., root tips were macerated in a 1:1 mixture of 45% acetic acid and 1 M hydrochloric acid at 60°C for 3 min., and then stained with 1% aceto-orcein. To study meiosis, young calathides of the three taxa were fixed in Carnoy's solution (3:1 ethanol:acetic acid) in the field, then transferred to 70% ethanol and refrigerated. Squashes were made in a drop of aceto-orcein.

For study of both ISSR markers and the sequences of the chloroplast *trnL-F* region, the young leaves of 56 individuals (20 of *Ligularia duciformis*, 20 of *L. paradoxa*, and 16 of the putative natural hybrid) were collected and dried in silica gel in the field. Total genomic DNA was extracted from the silica gel–dried leaf tissue using CTAB methods (Doyle & Doyle, 1987). All 90 ISSR primers were used for screening all individuals, and 10 primers (University of British Columbia, Canada [UBC] primers set #9: 807, 808, 809, 815, 820, 823, 827, 828, 834, 848) were selected for amplifying bands. The polymerase chain reaction (PCR) included the following cycles: preheat, 94°C, 5 min.; 45 cycles, denaturation, 94°C, 1 min.; annealing, 52°C, 1 min.; extension, 72°C, 1.5 min.; final extension, 72°C, 7 min. The amplified bands from three taxa were scored as (1) common to both putative parents, (2) shared with one of the putative parents, or (3) unique to the putative natural hybrid. The chloroplast *trnL-F* region was amplified using the universal primers *trnC* and *trnF* (Taberlet et al., 1991). Amplification conditions were as follows: 1 cycle, 94°C, 4 min.; 28 cycles, 94°C, 45 sec.; 58°C, 45 sec.; 72°C, 2 min., followed by 1 cycle, 72°C, 8 min. The PCR products were purified by electrophoresis through a 1.2% agarose gel followed by use of an E.Z.N.A. Gel Extraction Kit (Omega, Guangzhou, China). All accessions were subjected to chloroplast *trnL-F* sequencing, with amplification primers in an ABI 3700 DNA automated sequencer with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California, U.S.A.). Boundaries were determined by comparison with *Nicotiana paniculata* L. (AY098701). All sequences have been deposited in GenBank (accession numbers DQ104429–DQ104431). The sequences were aligned and compared in SeqMan (DNASTAR, Beijing, China).

Results

Morphological Analysis

Ligularia duciformis and *L. paradoxa* belong to series *Retusae* S. W. Liu (Liu, 1989). The main morphological differences between the two species are leaf form and pappus color (Table 1, Fig. 1). The

Table 1. Morphological comparison of *Ligularia paradoxa*, *L. duciformis*, and the nothospecies *L.* ×*maoniushanensis*.

Taxon	Characters			
	Habitat	Stems	Leaf blades	Pappus
L. paradoxa	perennial herbs	proximally glabrous, distally arachnoid-puberulous	orbicular or broadly ovate, palmatisect	brown, shorter than tubular corolla and longer than its tube
L. duciformis	perennial herbs	glabrous or distally short yellow pilose	reniform or cordate, margin irregularly dentate	white, as long as tube of tubular corolla
L. ×*maoniushanensis*	perennial herbs	glabrous or sparsely pubescent above	broadly ovate, palmately parted	wine-colored, white at base, shorter than tubular corolla and longer than its tube

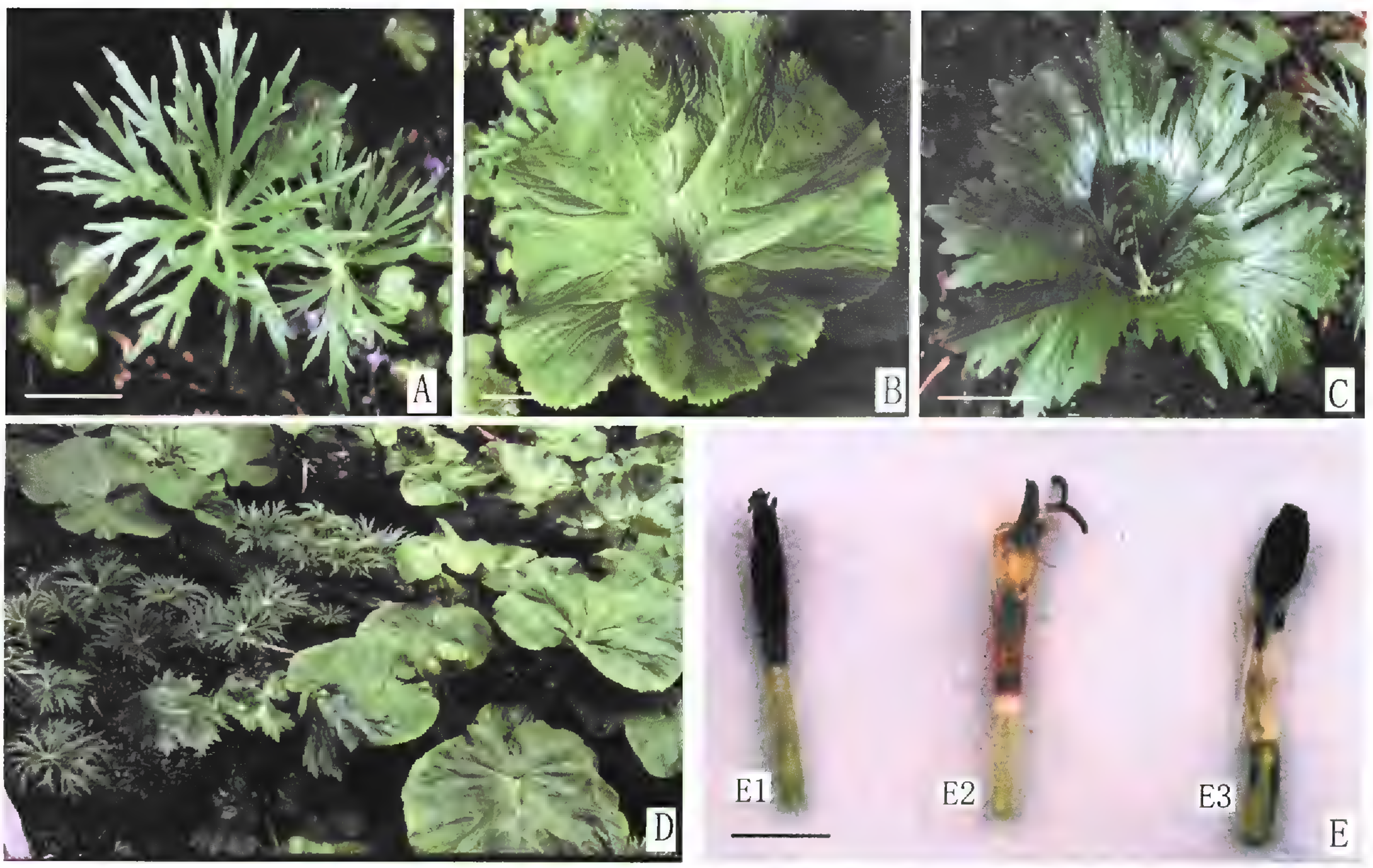

Figure 1. Photos of leaves, florets, and chromosomes of *Ligularia paradoxa*, *L. duciformis*, and *L.* ×*maoniushanensis*. —A. Leaf of *L. paradoxa*. —B. Leaf of *L. duciformis*. —C. Leaf of *L.* ×*maoniushanensis*. —D. *L. paradoxa*, *L. duciformis*, and *L.* ×*maoniushanensis* in a mixed population. —E. Florets of *L. paradoxa* (E1), *L. duciformis* (E3), and *L.* ×*maoniushanensis* (E2). —F. Somatic chromosomes of *L. paradoxa*. —G. Somatic chromosomes of *L. duciformis*. —H. Somatic chromosomes of *L.* ×*maoniushanensis*. Scale bars: A–C = 45 mm, E = 4 mm, F–H = 10 μm.

Table 2. The weight per 1000 seeds and germination rates of *Ligularia paradoxa*, *L. duciformis*, and the nothospecies *L.* ×*maoniushanensis*.

	L. paradoxa	*L.* ×*maoniushanensis*	*L. duciformis*
Weight per 1000 seeds (g)	1.17	0.79	2.26
Germination rate (%)	9.0	0	56.5

leaves of *L. paradoxa* are 3- to 8-palmatipartite (Fig. 1A, D), and those of *L. duciformis* are cordate or reniform with serrate margins (Fig. 1B, D). The pappus is white in *L. duciformis* (Fig. 1E3), and brown in *L. paradoxa* (Fig. 1E1). The morphological characters of leaf form and pappus of the putative natural hybrid are as follows: dissected peltate leaves with eight to 15 lobes (Fig. 1C, D) and wine-colored pappus (Fig. 1E2). The putative natural hybrid shares the morphological characters of its two putative parents in leaf form and pappus color.

SEED VIGOR

Embryos were observed in seeds of both *Ligularia paradoxa* and *L. duciformis*. No embryo was observed in the seeds of the putative natural hybrid. The weight and germination rates per 1000 seeds are listed in Table 2. There are distinct differences among the three taxa in the weight per 1000 seeds and in seed germination rates. No germination was observed for seeds of the putative natural hybrid, with a sample size of 1000 seeds investigated.

SOMATIC CHROMOSOME NUMBERS

The two putative parents and the putative natural hybrid are diploid and share the same chromosome number, $2x = 58$ (Table 3, Fig. 1F–H). Although the karyologies of the two putative parents are not well investigated, it is possible that hybridization takes place among taxa sharing the same chromosome number.

MEIOTIC BEHAVIOR

Chromosomes for the two putative parents displayed a normal meiotic process, characterized by the formation of 29 bivalents in diakinesis (Fig. 2A, D), with chromosome migration to two poles in anaphase I (Fig. 2B, E), and the formation of tetrads (Fig. 2C, F). However, the putative natural hybrid showed meiotic abnormalities in almost all phases, especially in diakinesis of meiotic prophase I with univalents, in anaphase I and II with lagged univalents and chromatid bridges, and in telophase II with free chromosomes (Fig. 2G–J).

ISSR MARKERS AND *TRNL-F* SEQUENCING

Although clear bands were amplified from three taxa using 10 primers, banding patterns of only four primers showed differences between the putative parents. The banding patterns of ISSR are summarized in Table 4. For example, a total of 13 bands were amplified from three taxa using the primer 807. Of these, nine bands were common to both putative parents, two bands were unique to *Ligularia paradoxa*, and two bands were unique to *L. duciformis*. The putative natural hybrid shared 12 bands with the putative parents, and no additive bands were observed (Fig. 2K).

The sequences of the chloroplast *trnL-F* region generated from the *Ligularia* hybrid and its parents were consistently 852 bp in length. No intraspecific sequence variation was observed for either parental species, although there were three nucleotide substitutions between the parental species (Table 5). All accessions of the putative hybrid had identical sequences with *L. paradoxa*. Thus, *L. paradoxa* is considered the female parent of this natural hybrid because chloroplast DNA is maternally inherited in *Ligularia* (Zhang et al., 2003).

Discussion

Speciation by hybridization has been well documented in plants (Abbot, 1992; Arnold, 1992;

Table 3. Chromosome numbers and meiotic behavior in *Ligularia paradoxa*, *L. duciformis*, and the nothospecies *L.* ×*maoniushanensis* from Mt. Maoniu, Ninglang Co., Yunnan, China.

Taxon	Voucher and origin	Chromosome number	Meiotic behavior
L. paradoxa	*Y. Gong 22356* (KUN)	$2n = 58$	Normal
L. duciformis	*Y. Gong 22355* (KUN)	$2n = 58$	Normal
L. ×*maoniushanensis*	*Y. Gong 22357* (KUN)	$2n = 58$	Abnormal

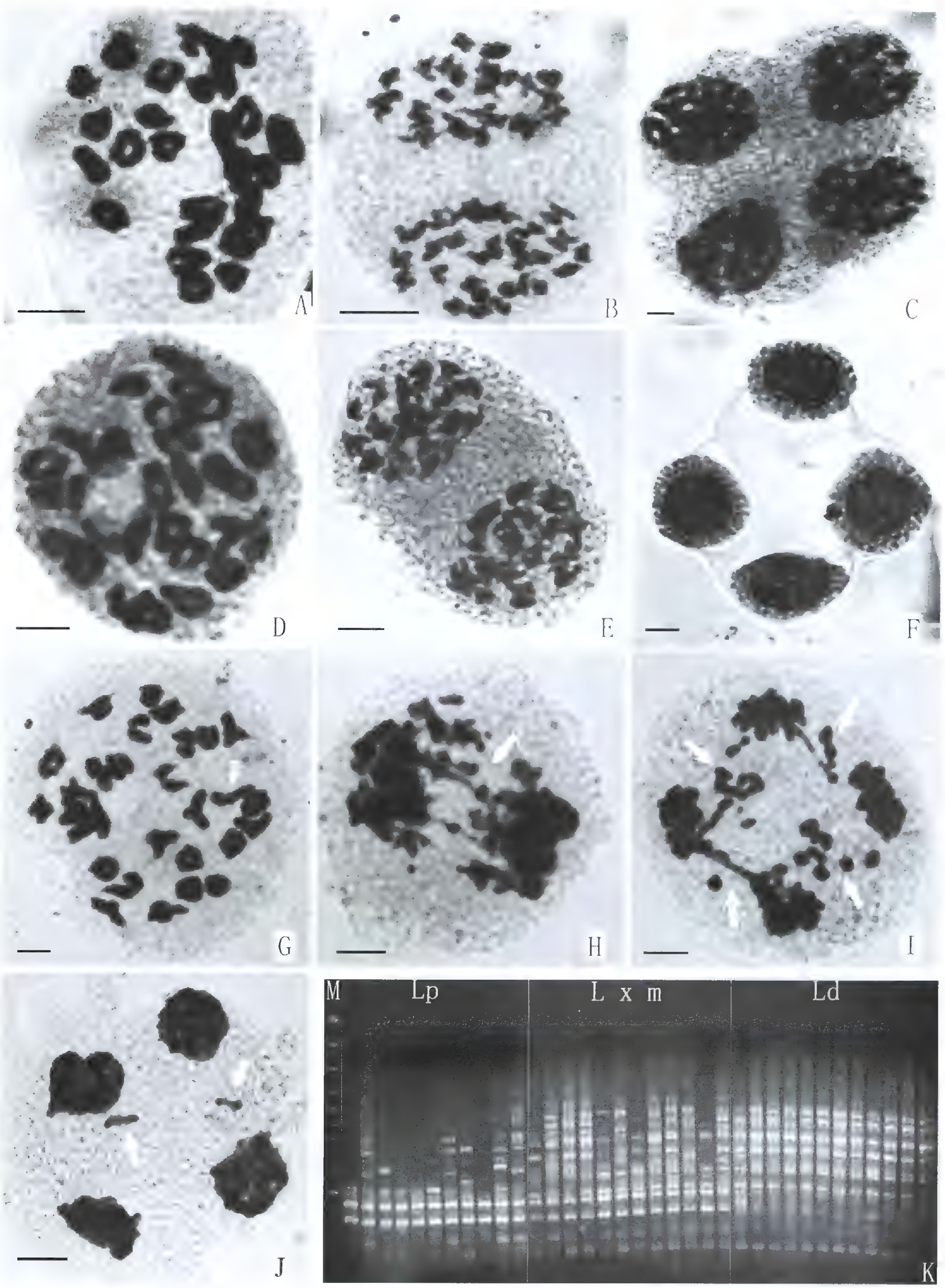

Figure 2. Meiosis of *Ligularia paradoxa*, *L. duciformis*, and the nothospecies *L.* ×*maoniushanensis*. A–C. *Ligularia paradoxa*. —A. Diakinesis. —B. Anaphase I. —C. Tetrad. D–F. *Ligularia duciformis*. —D. Diakinesis. —E. Anaphase I. —F. Tetrad. G–J. *Ligularia* ×*maoniushanensis*. —G. Diakinesis with univalents. —H. Anaphase I with lagged univalents and chromatid bridges. —I. Anaphase II with lagged univalents and chromatid bridges. —J. Tetrad with free chromosomes. —K. Banding ISSR patterns from primer 807 in *L. paradoxa* (Lp), *L. duciformis* (Ld), the nothospecies *L.* ×*maoniushanensis* (L × m), and marker banding (M). Scale bars: A–J = 10 μm.

Table 4. The banding patterns of ISSR amplified from the four primers 807(AGA GAG AGA GAG AGA GT), 823(TCT CTC TCT CTC TCT CC), 827(ACA CAC ACA CAC ACA CG), and 834(AGA GAG AGA GAG AGA G(CT)T) in *Ligularia paradoxa*, *L. duciformis*, and the nothospecies *L.* ×*maoniushanensis*. AB, number of ISSR bands additive to those for the putative natural hybrid; TB, total number of ISSR bands; UB, number of ISSR bands unique to one of the putative parents.

Taxon	Voucher and origin	ISSR primer														
		807			823			827			834			Total		
		TB	UB	AB	TB	UB	AB	TB	UB	AB	TB	UB	AB	TB	UB	AB
L. paradoxa	*X. Gong 22356* (KUN)	11	2	–	8	1	–	7	2	–	5	0	–	31	5	–
L. ×*maoniushanensis*	*X. Gong 22357* (KUN)	12	–	0	10	–	0	15	–	0	9	–	0	46	–	0
L. duciformis	*X. Gong 22355* (KUN)	11	2	–	9	2	–	13	8	–	9	4	–	42	16	–

Rieseberg, 1995). Gottlieb (1972) discussed several criteria for testing whether a particular diploid taxon originated through hybridization. These features included a geographical distribution in the region of parental sympatry, morphological intermediacy in several characters, partial fertility, biochemical additivity, and experimental synthesis of individuals resembling the hybrid. Although no single criterion can provide a clear means for testing a hypothesis of hybridization, each criterion that can be fulfilled provides a higher level of support for a hybrid origin (Gottlieb, 1972). It is also essential that hybrid criteria be evaluated carefully because morphological intermediacy and sterility are not invariably associated with hybrids (Rieseberg, 1995) and may result from entirely separate processes (Les & Philbrick, 1993). Nevertheless, Gottlieb's criteria (1972) provide a convenient avenue for discussing the putative natural hybrid between *Ligularia duciformis* and *L. paradoxa*.

In the present study, the morphological characters of leaf shape and pappus color were clearly intermediate with those of *Ligularia paradoxa* and *L. duciformis*, and the putative natural hybrid satisfies Gottlieb's (1972) criterion of morphological intermediacy. *Ligularia paradoxa*, one of the only two species with palmatipartite leaves in *Ligularia*, is restricted to Yunnan and Sichuan provinces, China, while *L. duciformis* is distributed in Yunnan, Sichuan, Hubei, Gansu, and Ningxia provinces, China (Liu, 1989; Liu et al., 1994). The two putative parents are sympatric species in geographical distribution.

The somatic chromosome numbers of the three taxa are identical ($2n = 58$). This number also agrees with reports from other species such as *Ligularia hookeri* (C. B. Clarke) Hand.-Mazz., *L. vellerea* (Franch.) Hand.-Mazz., *L. hodgsonii* Hook, and *L. kanaitzensis* (Franch.) Hand.-Mazz. (Gong et al., 2001). Therefore, the putative nothospecies is most likely a natural hybrid diploid. Both putative parents have displayed normal meiotic processes, producing seeds that germinated, suggesting that it is possible for hybridization to take place between *L. paradoxa* and *L. duciformis*.

ISSR markers are inherited in a dominant or codominant Mendelian pattern (Gupta et al., 1994; Tsumura et al., 1996) scored as diallelic, and present or absent. The absence of a band is interpreted as primer divergence or loss of a locus through the deletion of the simple sequence repeat site or chromosome rearrangement (Wolfe et al., 1998). In the present study, a total of 46 ISSR bands were amplified from three taxa using four primers. Twenty-six bands were common to both putative parents, and unique bands were distributed: five bands for *Ligularia paradoxa* and 16 bands for *L. duciformis*.

Table 5. Variable sites of the nucleotide sequences of chloroplast *trnL-F* region in *Ligularia paradoxa*, *L. duciformis*, and the nothospecies *L.* ×*maoniushanensis*.

Taxon (no. of individuals)	Voucher and origin	Variable site			GenBank accessions
		152	214	491	
L. duciformis (20)	*X. Gong 22355* (KUN)	T	T	C	DQ104429
L. paradoxa (20)	*X. Gong 22356* (KUN)	C	C	A	DQ104430
L. ×*maoniushanensis* (16)	*X. Gong 22357* (KUN)	C	C	A	DQ104431

No additional bands were observed for the putative natural hybrid, but all 46 common bands occurred in the putative natural hybrid. Therefore, ISSR markers clearly indicate that the putative natural hybrid shares a relationship with both *L. paradoxa* and *L. duciformis*.

Judging from the chromosomal behavior in meiosis and the absence of seed vigor, the putative natural hybrid may not produce sexual offspring and introgression is not likely among the three taxa. No asexual reproduction of the putative natural hybrid was noted in the field, but individuals of the putative natural hybrid at various ages were observed in the mixed population. Thus, hybridization between *Ligularia paradoxa* and *L. duciformis* has occurred more than once as observed in their zone of contact, when populations co-occur. In conclusion, the putative natural hybrid found on Mt. Maoniu is well supported as an interspecific hybrid between *L. paradoxa* and *L. duciformis*, and *L. paradoxa* is the female parent.

Taxonomic Treatment

Ligularia ×maoniushanensis X. Gong & Y. Z. Pan, nothosp. nov. ♀*Ligularia paradoxa* Hand.-Mazz. × ♂*Ligularia duciformis* (C. Winkl.) Hand.-Mazz. TYPE: China. NW Yunnan: Ninglang Co., Mt. Maoniu, 4200 m, 16 July 2001, *X. Gong 22357* (holotype, KUN!; isotype, MO not seen).

Hybrida naturalis e *Ligularia paradoxa* Hand.-Mazz. et *L. duciformis* (C. Winkl.) Hand.-Mazz. genita, ab ambabus laminis palmatifidis, magnitudine inter parentes mediis, pappis corollae vinaceis sed basi albis, seminibus abortivis facile differt.

Herbs perennial; stems simple, stout, 1.2–1.5 m tall, glabrous or sparsely pubescent above; basal and lower cauline leaves peltate, broadly ovate, 30–40 × 45–52 cm, 7- to 12-palmatipartite, 5- to 8-lobed, abaxially fulvous pubescent; 7 to 12 nerves, petioles ca. 30 cm, winged, clasping at base; upper leaves smaller. Inflorescence corymbose, peduncle 2–9 cm, densely fulvous pubescent, involucre campanulate, 10–12 × 2–3 mm, bracts in 2 series, 8 to 10 bracts, narrowly lanceolate, acute, externally densely fulvous pubescent. Ray florets absent; disc florets 5 to 7, bisexual, corolla tubular, 8–12 mm. Achenes cylindrical, glabrous; pappi wine-colored, white at base. Chromosome number $2n = 58$.

Distribution. Known only from the type specimen collected from Mt. Maoniu.

Habitat. The putative natural hybrid occurs within a mixed population of *Ligularia paradoxa* and *L. duciformis* within an area of about 2 km² along a stream bank in spruce-fir forest about 4200 m altitude on Mt. Maoniu in Ninglang, Yunnan, China. When we collected the specimen for the first time in July 2001, we observed 122 individuals, 116 in October 2001, and 130 in early August 2002.

IUCN Red List category. We propose that *Ligularia ×maoniushanensis* be recognized as Endangered (EN), according to IUCN Red List criteria, because of the small population size (< 250 mature individuals) in a small distribution area (< 2 km²) (IUCN, 2001).

Phenology. Observed as flowering from July to August; fruiting from September to October.

Paratype. CHINA. **Yunnan:** Ninglang Co., Mt. Maoniu, 4200 m, 23 July 2003, *Q. T. Zhang, Y. Z. Pan & Z. Y. Yang 082306* (KUN).

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A REVIEW OF THE GENUS *PYROSTEGIA* (BIGNONIACEAE)[1,2]

Amy Pool[3]

Abstract

Pyrostegia C. Presl is a genus of four species in the tribe Bignonieae. All the species are lianas with compound leaves with two leaflets and a terminal tendril, campanulate calyces, narrow corollas with lobes valvate basally in bud, four exserted stamens, compressed, linear capsules that dehisce parallel to the septum, and thin, bialate seeds. Three of the species are apparently hummingbird pollinated and have flowers that are very similar in appearance with red-orange (rarely yellow), narrow tubular-infundibular corollas. The fourth species, which is probably moth pollinated, has strongly fragrant, white, salverform corollas. All four species are native to South America. *Pyrostegia venusta* (Ker Gawl.) Miers, a popular ornamental, is cultivated throughout the tropics. Lectotypes are designated for *Bignonia ignea* Vell., *B. tecomiflora* Rusby, *P. cinerea* Bureau ex K. Schum., *P. dichotoma* Miers ex K. Schum., *P. venusta* var. *villosa* Hassl., and *Tynanthus igneus* Barb. Rodr. A key to the species, species descriptions, and a species distribution map are provided, and the relationships of the species are discussed.

Key words: *Arrabidaea*, Bignoniaceae, Bignonieae, hummingbird pollination, *Pyrostegia*.

Pyrostegia C. Presl is a small but diverse genus of four species in the tribe Bignonieae (Bignoniaceae). The genus is native to South America, with *P. venusta* (Ker Gawl.) Miers frequently cultivated throughout the tropics. Like most members of the tribe Bignonieae, *Pyrostegia* species are lianas with the terminal leaflet of their compound leaves usually modified as a tendril, and the fruits dehiscent parallel to the septum. All the species of *Pyrostegia* have stems with multiples of four phloem wedges (Santos, 1995), leaves with two leaflets and a terminal tendril, calyces campanulate with five minute denticules, fairly narrow, moderately thick-textured corollas with the narrow lobes valvate at their bases in bud, four exserted stamens, compressed, linear capsules, and thin, bialate seeds. Three of the species are apparently hummingbird pollinated and have flowers that are very similar in appearance: red-orange (rarely yellow), narrow tubular-infundibular corollas with the stamens inserted below the middle of the corolla tube. However, two of these species (*P. dichotoma* Miers ex K. Schum. and *P. venusta*) have nodes with glandular fields absent, tendrils apically trifid, and the corolla tubes externally glabrous, while the third species (*P. cinerea* Bureau ex K. Schum.) has nodes with glandular fields present, simple tendrils, and densely pubescent corolla tubes. The presence or absence of interpetiolar glandular fields is a useful character in recognizing genera in the Bignonieae (Sandwith, 1938; Seibert, 1948; Gentry, 1993) and has frequently been used as a generic criterion. However, Gentry (1980) warned that the presence of interpetiolar glandular fields can be variable even within a species and should not be used alone as an absolute generic character. Similarly, leaf tendrils can be an extremely useful generic character, with some genera having only simple tendrils, while other genera have only trifid tendrils (Urban, 1916; Gentry, 1993). However, there are genera and even species where both types of tendrils are found (Gentry, 1980), so again, this character should not be used as an absolute in defining genera. Finally, corolla pubescence can also be an important character for differentiating genera (Gentry, 1980) and is frequently employed in keys to genera (Sandwith, 1938; Gentry, 1982), but again, it can be variable within a genus. Nonetheless, retaining *P. cinerea* within the genus *Pyrostegia* is somewhat suspect, and alternative placement is discussed in the taxonomy section of this paper. The fourth species in the genus (*P. millingtonioides* Sand-

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with) has nodes with glandular fields absent, tendrils apically trifid, and the corolla tubes externally glabrous, but has flowers that are quite different in appearance from the other three species. It has strongly fragrant, white, salverform corollas with stamens inserted near the mouth of the corolla tube and is probably moth pollinated. Gentry (1990a: 125) found that switches from one pollinator to another within a genus are relatively rare and suggested that "such changes appear mostly to be associated with major genetic reorganization that accompanies generic differentiation." Placement of *P. millingtonioides* is discussed in more detail in the taxonomy section of this paper.

History

C. Presl established the genus *Pyrostegia* (Presl, 1845). He provided a thorough description of the genus and the type species, *Bignonia ignea* Vell. (= *P. venusta*), which he transferred into his new genus. However, he made no mention of the aestivation of the corolla lobes, a character made much of by other authors. De Candolle (1845) had treated *B. ignea* as a synonym of *B. venusta* Ker Gawl., placing emphasis in his description on the valvate aestivation of the corolla lobes. Miers (1863: 188) also emphasized the corolla lobe aestivation, describing the lobes as "almost valvate in aestivation, a feature quite peculiar to the genus." Miers (1863) transferred *B. venusta* and *B. longiflora* Cav. to *Pyrostegia* and listed 10 additional names as new species, but did not provide descriptions for the new taxa. Schumann (1894) established the new genus *Macranthisiphon* Bureau ex K. Schum., into which he transferred *B. longiflora*. Monotypic, *Macranthisiphon* is similar to most of the species of *Pyrostegia* in having narrow, orange-red corollas with narrow corolla lobes and exserted stamens; it differs in the corolla lobes being imbricate and in having large foliaceous pseudostipules. Schumann (1894) also published two more species of *Pyrostegia*, *P. cinerea* and *P. dichotoma*, in his treatment of Bignoniaceae for *Die Natürlichen Pflanzenfamilien*, and used the valvate aestivation of the corolla lobes as an early lead in his key to genera in the tribe Bignonieae. In their treatment of Bignoniaceae for *Flora Brasiliensis*, Bureau and Schumann (1896, 1897), treated *P. ignea* (Vell.) C. Presl as a synonym of *P. venusta*, added the new species *P. tubulosa* Bureau & K. Schum. (= *P. dichotoma*), and again emphasized the valvate aestivation of the corolla lobes by using this character to separate *Pyrostegia* and *Glaziova* Bureau from other genera of the Bignonieae with 3(2, 1)-foliolate leaves, filiform tendrils, and floral discs. Urban (1916) transferred *B. tecomiflora* Rusby (= *P. dichotoma*) to *Pyrostegia*. Sandwith added *P. millingtonioides* to the genus and, while commenting that his new species was unusual in the genus both in corolla color and shape and in the level of insertion of the stamens, nonetheless expressed that it could be placed in this genus "with some confidence" (Sandwith, 1962: 465). This was the last addition to the genus. Macbride (1961) placed *P. dichotoma* in synonymy with *P. venusta* in the *Flora of Peru*, but Gentry (1982) recognized the two as close but separate species in his treatment of Bignoniaceae for the *Flora de Venezuela*.

Anatomy

Santos (1995) studied 31 genera of Bignonieae and divided them into four groups based on the type of cambial variant. Three species of *Pyrostegia*, *P. cinerea*, *P. dichotoma*, and *P. millingtonioides*, were included in her study. *Pyrostegia* falls into her group 2, which is characterized by possession of stems with multiples of four phloem wedges. Santos found all the genera in her group 2 to be so similar that it was difficult to separate them anatomically. All the genera in this group were found to have septate fibers, cylindrical stems and absence or rare occurrence of perforated ray cells, and scanty to vascicentric paratracheal parenchyma. Other genera in group 2 are *Amphilophium* Kunth, *Anemopaegma* Mart. ex Meisn., *Clytostoma* Miers ex Bureau, *Cydista* Miers, *Distictis* Mart. ex Meisn., *Haplolophium* Cham., *Mansoa* DC., *Mussatia* Bureau ex Baill., *Periarrabidaea* A. Samp., *Phryganocydia* Mart. ex Bureau, *Roentgenia* Urb., and *Tanaecium* Sw.

Palynology

The pollen of *Pyrostegia* is 3-colpate (or sometimes 4-colpate in *P. venusta*) and finely reticulate (Urban, 1916; Sandwith, 1962; Gentry & Tomb, 1979), a pollen type common in the tribe Tecomeae and found in several probably unrelated genera in the Bignonieae (Gentry & Tomb, 1979). Other genera with similar pollen in the Bignonieae include *Roentgenia* (ca. 4-colpate, and exine somewhat warty), *Potamoganos* Sandwith, *Stizophyllum* Miers, *Pachyptera* DC. ex Meisn., and some species of *Tanaecium* (Gentry & Tomb, 1979). In *Pyrostegia*, only *P. dichotoma* and *P. venusta* have been examined using SEM (Gentry & Tomb, 1979: fig. 3).

Cytology

Goldblatt and Gentry (1979) found a base number of $x = 20$ for all but three of the 23 genera of the tribe Bignonieae known cytologically. Their count for

Pyrostegia cinerea (voucher: *Gentry 12773*) of $2n = 40$ is consistent with this general tribal base number. Joshi and Hardas (1956) reported a count of $3n = 60$ for *P. ignea* (= *P. venusta*) from a non-fruit-setting population cultivated in India. Other species (and populations) of *Pyrostegia* have not been investigated cytologically.

Reproductive Biology

Flowers of *Pyrostegia cinerea*, *P. dichotoma*, and *P. venusta* are typical of those pollinated by hummingbirds: odorless and the corolla usually bright red-orange, of fairly thick texture, with a narrow tube and wider mouth, and more or less glabrous internally (Gentry, 1980, 1990a), while the aromatic flowers of *P. millingtonioides*, with white, narrow, tubular, and thickly textured corollas, are probably moth pollinated (Gentry, 1980, 1990a). Hummingbirds have been reported visiting *P. venusta* (Gobatto-Rodrigues & Stort, 1992; Galetto et al., 1994). Gobatto-Rodrigues and Stort (1992) found *P. venusta* to be self-compatible with facultive outbreeding. The hummingbirds *Eupetomena macroura* Gmelin and *Phaethornis pretrei* Lesson & Delattre were reported as visiting and effectively pollinating *P. venusta*, while a number of insects were found to visit the flowers as nectar or pollen thieves. Galetto et al. (1994) reported the hummingbirds *Chlorostilbon aureoventris* Orbigny & Lafresnaye and *Sappho sparganura* Shaw visiting flowers of *P. venusta*. Their study illustrated the pattern of nectar secretion in *P. venusta*. Gusman and Gottsberger (1996) compared the usual orange-flowered *P. venusta* with the rarer yellow-flowered individuals in respect to floral morphology, nectar composition, carotenoids, and flavenoids, and theorized about how these differences could affect nectar robbing by bees and visits by hummingbirds.

Gentry (1990a) hypothesized that all moth- and hummingbird-pollinated species of Bignoniaceae bloom for a period of several weeks to a few months, building up to a distinct peak of flower production followed by a gradual decrease in number of flowers. He referred to these as cornucopia species. Galetto et al. (1994) and Gobatto-Rodrigues and Stort (1992) confirmed this pattern for the populations of *Pyrostegia venusta* observed in their studies, but with the flowers being produced over a time span of several months (Galetto et al., 1994) or generally for about six months with a two-month peak (Gobatto-Rodrigues & Stort, 1992). This herbarium study did not prove particularly useful in confirming the patterns of phenology. Only two flowering specimens of *P. millingtonioides* were seen, these collected in July and August. Six flowering collections of *P. cinerea* were observed: May (1), June (2), July (2), and November (1). *Pyrostegia venusta* has been seen in flower throughout the year (except December) but peaks between June and September. *Pyrostegia dichotoma* is more complicated. For its whole range, it has been seen in flower all year, but in Colombia only in January; in Guyana and Suriname in February, September, and October; in Venezuela peaking in March; and in Peru, Bolivia, and Brazil peaking from June to October.

Economic Uses

Pyrostegia venusta is a very popular ornamental, cultivated throughout the tropics. Menninger et al. (1970) included it (as *P. ignea*) on his list of the most beautiful flowering climbers in the world and ranked it as the most popular of all in the tropics.

Materials and Methods

Gentry established a private database that compiled label information from herbarium specimens at MO and other herbaria that he examined personally. Gentry's private database has been incorporated into the Missouri Botanical Garden database-management system TROPICOS, <http://www.tropicos.org/>, which now contains label information for all *Pyrostegia* specimens housed at MO in addition to those received on loan from AAU, B, BM, BR, C, CAS, CM, F, FTG, G, GH, GOET, L, M, MICH, NY, S, TEX, U, UPS, US, and W. Specimens determined by this author were selected from this database to generate the Index to numbered exsiccatae (Appendix 1) and the distribution maps, the latter with the assistance of Duan Bills. Dots on the maps (Fig. 1) represent a subset of the specimens examined by this author, including specimens with geographic coordinates provided on collection labels and a set of specimens, for which the author estimated the geographic coordinates, selected to illustrate the full geographic range for each species.

Taxonomic Treatment

Pyrostegia C. Presl, Abh. Königl. Böhm. Ges. Wiss., ser. 5, 3: 523. 1845. TYPE: *Pyrostegia ignea* (Vell.) C. Presl [Based on *Bignonia ignea* Vell.].

Lianas; branchlets subangulate with 6 to 8 inconspicuous ribs, lacking, or less frequently with, interpetiolar glandular fields, not lenticular; pseudostipules usually inconspicuous, small and subulate. Leaves 2(3)-foliolate, often with a tendril; tendril filiform, apically trifid or less frequently simple, deciduous. Inflorescence a terminal or axillary pani-

cle; bracteoles minute, subulate, and inconspicuous; flowers usually numerous. Flowers with the calyx campanulate, truncate to undulate, the ribs extending apically to form 5 small denticules, these sometimes inconspicuous; corolla narrow tubular-infundibular, moderately thick, red-orange (rarely yellow), and odorless or, less frequently, salverform, thick, white, and fragrant; tube externally glabrous, or less frequently densely pubescent throughout, internally glabrous except at, and sometimes, below the level of insertion of stamens; lobes narrow, ± valvate at base in bud, pubescent at least along margins; stamens 4, exserted, anthers glabrous, pendulous, dithecal, the thecae subparallel to divaricate; staminode present, small; disc annular-pulvinate; ovary linear-tetragonal, lepidote, bilocular, placentation axile, ovules biseriate in each locule; stigma exserted, broadly, or less frequently, narrowly, bilamellate. Fruit compressed, linear, the valves parallel to the septum, smooth, medial vein sometimes slightly raised; seeds thin, bialate, wings brown with hyaline margins.

Key to Species of *Pyrostegia*

1. Corollas white, narrowly salverform, ca. 3 mm wide at mouth of tube, lobes elliptic-oblong, at least 1/2 as wide as long, 0.6–0.7 cm long; stamen filaments 0.15–0.2 cm long; interpetiolar ridge present . . . . . 3. *P. millingtonioides*

1′. Corollas red-orange (rarely yellow), narrowly tubular-infundibular, 6–13 mm wide at mouth of tube, lobes oblong, less than 1/2 as wide as long, 1–1.8 cm long; stamen filaments 2.6–5.5 cm long; interpetiolar ridge absent.

2. Corolla tube externally densely red-puberulent; leaflets with pubescence densely silvery- or ferruginous-tomentose, adaxially glabrescent; tendrils simple; interpetiolar glandular fields present; stigma lobes subulate . . . . . 1. *P. cinerea*

2′. Corolla tube externally glabrous; leaflets glabrous to densely short-pilose; tendrils apically trifid; interpetiolar glandular fields absent; stigma lobes orbicular, ovate, or broadly oblong.

3. Staminodes inserted at same level as stamens (rarely to 0.4 cm above insertion of stamens); inflorescence trichomes minute, appressed to ascending; inflorescence generally open; mature fruits generally drying brown to dark brown . . . . . 2. *P. dichotoma*

3′. Staminodes inserted (rarely 0.8)1.2–1.6 cm above insertion of stamens; inflorescence trichomes longer, initiating at right angles to surface; inflorescence generally crowded with calyces overlapping in dried specimens; mature fruits generally drying with olive cast . . . . . 4. *P. venusta*

1. **Pyrostegia cinerea** Bureau ex K. Schum. in Engl., Nat. Pflanzenfam. 4(3b): 223. 1894. TYPE: Brazil. Minas Gerais [cultivated at Rio de Janeiro: Quinta da Boa Vista], s.d. [28 Feb. 1882], *A. Glaziou 14124* (lectotype, designated here, P [barcode P00481542] not seen, P digitized image!; duplicates, P [barcode P00481543] not seen, P digitized image!, C!, photo F neg. 22143!).

Tynanthus igneus Barb. Rodr., Vellosia, ed. 2, 1: 50, 3: ser. 2: tab. 10. 1891, non *Pyrostegia ignea* (Vell.) C. Presl, 1845. TYPE: Brazil. Amazonas: "Arvensis ad ripas Rio Negro, prope Manaos," ser. 2, tab. 10 in Barbosa Rodrigues, Vellosia, ed. 2, vol. 3 (lectotype, designated here, tab. 10 in Barbosa Rodrigues, 1891b!).

Branchlets silvery- to ferruginous-tomentose, interpetiolar glandular fields present, interpetiolar ridge absent. Leaves 2-foliolate with a simple terminal tendril; petioles 0.5–2.2 cm, tomentose; petiolules 0.5–1.2 cm; leaflets oblong or ovate, subinequilateral, 4–12.5 × 2.5–6.5 cm, membranous, 4 or 5 pairs of lateral veins prominent below, silvery- to ferruginous-tomentose and adaxial surface eventually glabrescent, scattered pellucid-lepidote, base rounded, apex acute-mucronulate or briefly acuminate-mucronulate. Panicle terminal or terminal and axillary, narrowly elongate with calyces not overlapping in dried specimens, 1 to 3 times branched, peduncle, rachis, and bracteoles silvery- to ferruginous-tomentose; calyx excluding denticules 4–5 × 4–5 mm at apex, silvery- to ferruginous-tomentose, occasionally with few lepidote scales; corolla narrow tubular-infundibular, orange (rarely yellow); tube 2.5–3.5 cm long, 2.4–3 mm wide at base, 7–10 mm wide at mouth, internally sericeous at and below insertion of stamens, externally densely red-puberulent; lobes oblong, 1–1.5 × 0.3–0.4 cm, internally red-puberulent apically and marginally, externally densely red-puberulent; stamens and staminode inserted at approximately same level in corolla tube, 1–1.2 cm from base of tube, stamen filaments 2.7–3.2 cm, thecae porrect-divaricate, 2.3–3 mm, staminode 6–12 mm; disc 1.2–2 × 1.2–2 mm; pistil 4–5.5 cm, ovary 2–2.8 × 0.6–1 mm, stigma lobes subulate. Capsule 17.5–19 × 0.9–1 cm, glabrous, drying brown, midvein slightly raised, base and apex acute; seeds 0.7–0.9 × 2.2–2.5 cm (Fig. 2C–F).

Distribution. Brazil, disturbed areas or campinas (white sand areas) near Manaus, State of Amazonas, presumably at low elevations (Fig. 1).

Phenology. Flowering May to July and November; fruiting November.

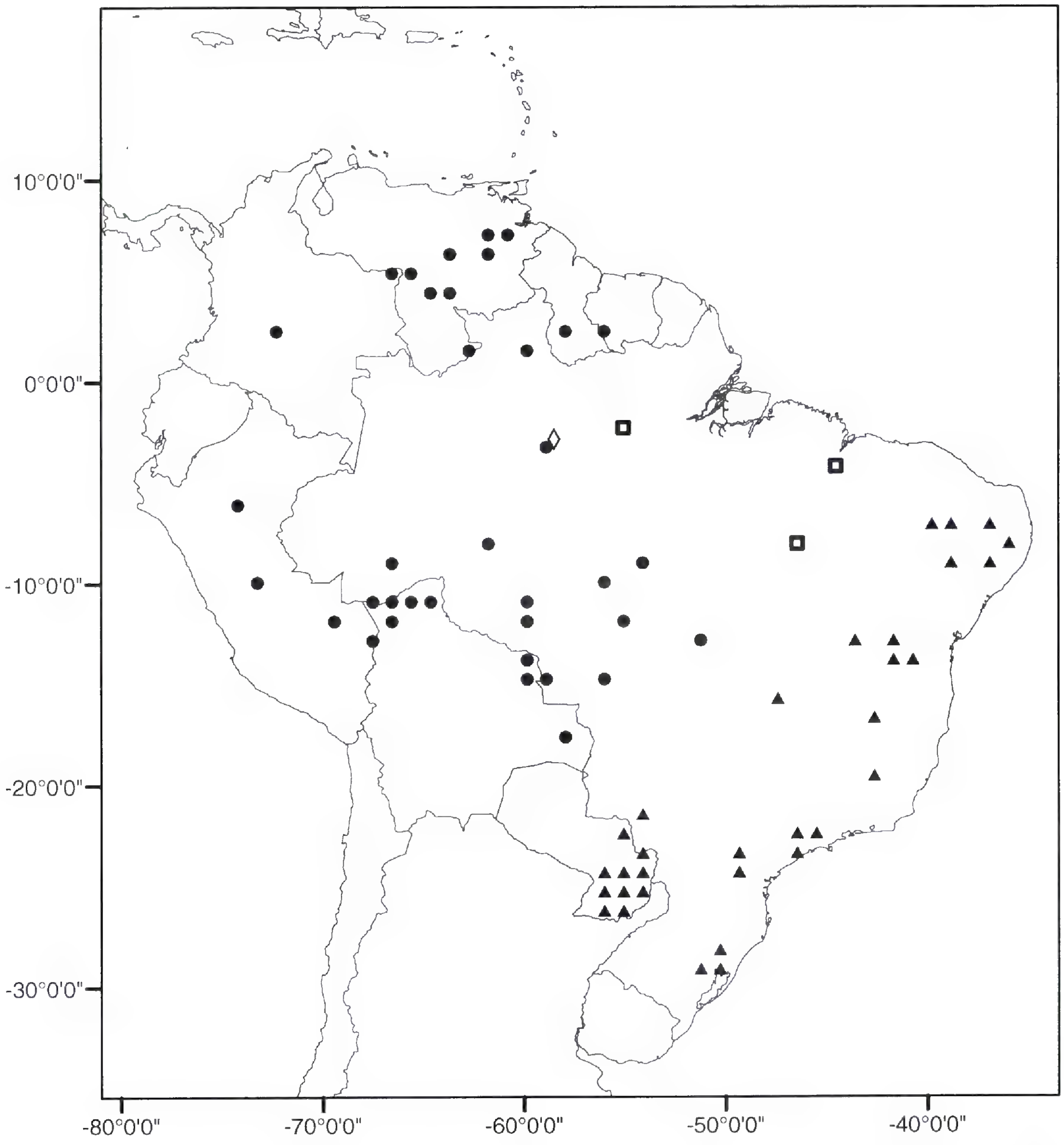

Figure 1. Distribution of species of *Pyrostegia*, excluding cultivated and apparently naturalized specimens. *Pyrostegia cinerea* Bureau ex K. Schum. (◇), *P. dichotoma* Miers ex K. Schum. (●), *P. millingtonioides* Sandwith (□), and *P. venusta* (Ker Gawl.) Miers (▲).

Discussion. Schumann (1894) published *Pyrostegia cinerea* Bureau ex K. Schum. in his key to *Pyrostegia* without any indication of type collection. It is assumed here that the species was included in Schumann's key based on information from Bureau and that the type collection was at P. Two collections at P are annotated in what I believe to be Bureau's handwriting. *A. Glaziou 14124* (barcode P00481542) is annotated by Bureau as "Nouveau," and with potential specific epithets (discolor and cinerascens) with a line drawn through them and finally "*cinerea* α discolor." *Jobert 452* is annotated by Bureau as "*Pyrostegia cinerea* n. sp. β concolor." The *Glaziou* collection is chosen here as the lectotype in part because I suspect that Bureau would have been more likely to indicate the type variety as variety α versus variety β, and because duplicates of *Glaziou 14124* are extant; in fact the sheet at C also seems to be annotated in Bureau's hand. Unfortunately, while *P. cinerea* is endemic to Amazonas, Brazil, the lectotype is labeled as from Minas Gerais. However, Glaziou is known to have changed localities (and sometimes also the collector, collection number, and/or date of collection) on some specimens (Wurdack, 1970).

Figure 2. A, B. *Pyrostegia millingtonioides* Sandwith (from *G. S. Pinheiro 19*, MO). —A. Flower bud. —B. Longitudinal section of corolla with androecium, style, and stigma. C–F. *Pyrostegia cinerea* Bureau ex K. Schum. (from *A. Ducke 532*, MO). —C. Flowering branch. —D. Stem node with glandular field. —E. Style and stigma. —F. Longitudinal section of corolla with androecium.

The duplicate of *A. Glaziou 14124* at C is without locality information. The second collection of *A. Glaziou 14124* at P (barcode P00481543) is labeled as made from cultivated material at Quinta da Boa Vista, Rio de Janeiro, collected 28 Feb. 1882. Glaziou (1911) reports both his numbers *14124* and *19666* (the latter cited by Bureau & Schumann, 1897, as *19665*, from Minas Gerais) as cultivated at Quinta da Boa Vista, S. Christovão, Rio de Janeiro. It is therefore assumed that the lectotype *A. Glaziou 14124* (barcode P00481542), while labeled Minas Gerais, is a duplicate of *A. Glaziou 14124* (barcode P00481543), and was made from cultivated material at Quinta da Boa Vista, Rio de Janeiro. *A. Glaziou 14124* (barcode P00481543) is not a lectotype candidate as it was formerly in Glaziou's private herbarium, not donated to P until 1907, and not annotated by Bureau.

According to Stapf (1909: 225), Barbosa Rodrigues made his descriptions and illustrations from living plants with an "almost complete absence of specimens." Stafleu and Cowan (1983: 829) were unable to locate any types or other herbarium specimens of Barbosa Rodrigues and concluded that the most important protologue materials for Barbosa Rodrigues' new taxa were to be found in his published and unpublished drawings. Barbosa Rodrigues' excellent plate, ser. 2, tab. 10 (Barbosa Rodrigues, 1891b) is thus chosen here as the lectotype of *Tynanthus igneus*. The quality of the illustration and description leave no doubt that *T. igneus* is synonymous with *Pyrostegia cinerea*, a conclusion previously reached by Bureau and Schumann (1896: 198). While *T. igneus* Barb. Rodr. (Barbosa Rodrigues, 1891a) has priority over *P. cinerea* Bureau ex K. Schum. (1894), *P. ignea* (Vell.) C. Presl (1845) blocks the transfer of the Barbosa Rodrigues name to *Pyrostegia*.

The simple tendrils, interpetiolar glandular fields, and externally pubescent corollas of this species are unexpected in the genus *Pyrostegia*, where they are otherwise not known (Fig. 2C, D). These characters suggest an affinity with the genus *Arrabidaea* DC. Sandwith (1968: 405–406) established a set of characters, which when found in combination, could suggest inclusion in the genus *Arrabidaea*: "branchlets terete or subterete, not definitely tetragonous; tendrils simple; corolla tomentose outside, at least on the limb, nearly always pink, purple or white, very rarely creamy yellow, never full yellow; disk conspicuous; anther thecae parallel, divergent or divaricate, glabrous, not curved or contorted; pollen-grains simple, 3-furrowed; ovary oblong; ovules 2 to 4 seriate in each loculus; capsule narrow, linear-oblong, compressed. Glandfields may be present or absent at the nodes, though commonly present; trichomes of the indumentum may be short and simple, long and multicellular, sometimes gland-tipped, or branched and 'dendroid' (sometimes forming a dense fur), but not stellate; the foliage in two species is for the most part bipinnately 2–3 ternate; foliaceous pseudostipules are rarely, but sometimes present; the inflorescence is often a pyramidal thyrse, but may be short and axillary and is sometimes found on the old wood; the calyx is extraordinarily variable in shape, size and texture, and in the presence or absence of teeth or lobes; the corolla varies from hypocratiform in species with very small flowers to campanulate-funnelshaped in most of those with larger flowers; the stamens are usually included but in one species the anthers are conspicuously exserted; the capsule is usually smooth, but in two widely distributed species is densely prickly-tuberculate; the seeds usually have membranous wings, but in certain water-borne species are wholly corky." *Pyrostegia cinerea* differs from this typical picture of *Arrabidaea* in its large, orange, narrow tubular-infundibular corolla and exserted stamens. Sandwith's concept of *Arrabidaea* included no species with true yellow or orange corollas; however, *A. elegans* (Vell.) A. H. Gentry does have yellow to orange corollas, though Gentry (1975) suggests that this and *A. bilabiata* (Sprague) Sandwith might be better placed in a separate genus. *Arrabidaea lauta* Bureau & K. Schum. is very similar to *P. cinerea* and was initially placed in *Pyrostegia* by Miers (1863). Not only is it similar florally, with a pubescent, narrow tubular-infundibular corolla with oblong lobes and exserted stamens, but it is also similar vegetatively, with interpetiolar glandular fields and 2-foliolate leaves with a simple tendril. This might suggest that *A. lauta* would fit more comfortably in the genus *Pyrostegia*, rather than supporting a relationship between *P. cinerea* and the genus *Arrabidaea*. However, *A. lauta* differs from species of *Pyrostegia* in the imbricate aestivation of its corolla lobes, more delicately textured, red corolla, strongly recurved anther thecae, and relatively broader calyx. The pollen of *P. cinerea* was examined by Urban (1916) using light microscopy and found to be more similar to that of *P. dichotoma* and *P. venusta*, that is, 3(or 4)-colpate and finely reticulate (Gentry & Tomb, 1979), than to species of *Arrabidaea*, 3-colpate and more or less psilate or smooth (Gentry & Tomb, 1979). Anatomically, *P. cinerea* fits well with the other two species of *Pyrostegia* examined (Santos, 1995) into Santos' (1995) group 2, characterized by stems with multiples of four phloem wedges in transverse section. Species of *Arrabidaea* belong to Santos' (1995) group 1, characterized by stems with only four phloem wedges in transverse section. Crystals were found in the vessels of most of the species of

Arrabidaea examined, but not in any of the species of *Pyrostegia*.

Pyrostegia cinerea is maintained for the time being in *Pyrostegia* primarily on the basis of the corolla lobe aestivation, valvate for the basal half to two thirds. This appears to be an unusual character state in the Bignoniaceae. The narrow tubular-infundibular corolla shape found in *Pyrostegia* appears to have evolved repeatedly in the family and in the tribe Bignonieae in groups pollinated by hummingbirds (Gentry, 1974, 1980); however, this does not seem to be true of the corolla lobe aestivation. Gentry (1990a) lists the following genera of the Bignonieae as strictly hummingbird pollinated: *Dolichandra* Cham., *Fridericia* Mart., *Gardnerodoxa* Sandwith, *Macranthisiphon*, *Martinella* Baill., and *Piriadacus* Pichon. All of these have the corolla lobes clearly imbricate in bud.

Additional specimens examined. BRAZIL. Northern Brazil, *Jobert 452* (P not seen, P digitized image). **Amazonas:** Manaus, *C. A. W. Schwacke III-278* (GOET); Manaus, Ig. da Cachoeira Baixa do Tarumã, *J. Chagas s.n. INPA-1198* (U); Estrada de Aleixo, NE Manaus, ca. 10 km to Leper Colony, *A. Lasseigne P22582* (MO); along Aleixo-rd., *P. J. Maas & H. Maas 326* (MO); outskirts of Manaus, rd. to INPA, *A. H. Gentry 12857* (MO); Manaus, loco Estrada do Aleixo, *A. Ducke 532* (MO), *532-II* (US); grounds of INPA at Manaus, *A. H. Gentry & G. T. Prance 11198* (MO); vic. of Manaus rd. toward Rio Negro, 10 km N from Manaus on Estrada Aleixo, *A. H. Gentry et al. 12773* (MO); Km 14 on Estrada Mauá, *G. T. Prance & Prance 21012* (NY); Reserva Florestal Ducke, Manaus–Itacoatiara, Km 26, Floresta de Campinarana, *L. G. Lohmann et al. 34* (MO). **Rio de Janeiro:** [Cultivated at Quinta da Boa Vista, S. Christovão], *A. Glaziou 19666* (C, P not seen, P digitized image).

2. Pyrostegia dichotoma Miers ex K. Schum. in Engl., Nat. Pflanzenfam. 4(3b): 223. 1894. TYPE: Peru. San Martín: Tarapoto, 1855–1856, *R. Spruce 3930* (lectotype, designated here, G!, photo F neg. 26203!; isotypes, BM!, BR!, G-DC not seen, GH!, K not seen, NY!, P not seen, W!).

Bignonia tecomiflora Rusby, Mem. Torrey Bot. Club 6: 101. 1896, "*tecomaeflora*." *Pyrostegia tecomiflora* (Rusby) K. Schum. ex Urb., Ber. Deutsch. Bot. Ges. 34: 746. 1916. TYPE: Bolivia. La Paz: Mapiri, July–Aug. 1892, *M. Bang 1510* (lectotype, designated here, NY [barcode 278056]!; duplicates, A!, BM!, MICH!, MO!, NY [barcode 564088]!, US!).

Branchlets sparsely lepidote and/or sparsely to densely covered with small curved-ascending to appressed trichomes, or glabrous, interpetiolar glandular fields absent, interpetiolar ridge absent. Leaves 2-foliolate, often with an apically trifid terminal tendril, or leaves 3-foliolate; petioles 1.5–4 cm, with curving trichomes in adaxial canal; petiolules 0.5–3 cm; leaflets ovate, slightly subinequilateral, 2.5–12 × 1.4–6.7 cm, chartaceous (membranous), 3 to 5 pairs of lateral veins prominent abaxially, sometimes with trichomes along midvein on adaxial surface and/or at base of abaxial surface, pellucid-lepidote, often especially conspicuous abaxially, with large glands in the axils of lower lateral veins, base rounded or truncate, apex acuminate-mucronulate or briefly acuminate-mucronulate. Panicle usually terminal, sometimes axillary, open with calyces not overlapping in dried specimens, 2 to 4 times branched, peduncle, rachis, and bracteoles nearly glabrous to densely covered with minute, ascending-curved to appressed trichomes, sometimes also lepidote; calyx excluding denticules 4.5–8 × 4.5–10 mm at apex, with sparse lepidote scales, sometimes minutely puberulent, apex ciliate; corolla narrow tubular-infundibular, orange or reddish orange; tube 3.5–7 cm long, 3–5 mm wide at base, 6–13 mm wide at mouth, internally sericeous at and below insertion of stamens, externally glabrous; lobes oblong, 1–1.8 × 0.3–0.6 cm, puberulent apically and marginally, externally sometimes lepidote; stamens and staminode inserted at approximately same level in corolla tube (or staminode up to 4 mm above higher stamens), 1–3 cm from base of tube, stamen filaments 3.6–5.5 cm, thecae slightly divergent or subparallel, 3–4.5 mm, staminode (sometimes developing an anther) 2–25 mm; disc 1.5–2 × 2–3 mm; pistil 5–7 cm, ovary 3–7 × 1 mm, stigma lobes ovate, broadly ovate, or broadly oblong. Capsule (rarely 11)18–33 × 0.8–1.2 cm, glabrous, drying brown, midvein apparent but not conspicuous, base acute, apex aristate; seeds 0.6–1 × 2.6–4.5 cm (Fig. 3C–F; Gentry, 1982: fig. 38).

Distribution. Guyana, Suriname, Venezuela, Colombia, Peru, Bolivia, and Amazonian Brazil; 30–1500 m elevation, generally found below 1000 m. It is frequently reported to be found in disturbed areas, in savannas with islands of larger trees, or in dry, moist, or wet forest. The soil type is often described as sandy (Fig. 1).

Phenology. For whole range, flowering and fruiting throughout year. In Colombia flowering in January; in Guyana and Suriname in February, September, and October; in Venezuela peaking in March; and in Peru, Bolivia, and Brazil peaking from June to October.

Common names. Venezuela: Barqui (Gentry, 1982), Tango (*Xena 257*); Peru: Paccha huasca (*Ferreyra 5070*); Bolivia: Lluvio de Oro (*Guillén et al. 4108*).

Discussion. Miers (1863) published the name *Pyrostegia dichotoma*, without description, citing one collection, *Spruce 3930* from Tarapota [Peru]. Schumann (1894: 223) validly published *P. dichotoma* without directly specifying the type or any collections, but by citing "*Pyrostegia dichotoma* Miers aust Ostperu," indirectly indicated *Spruce 3930* as the

type. Bureau and Schumann (1897) cite the same collection as the only specimen of *P. dichotoma*. A number of specimens of this collection number were seen from a variety of herbaria (BM, BR, G, GH, NY, W, also at G-DC, K, and P, but these not seen), and it is assumed that Schumann also had a specimen at B, and that specimen was the holotype and is no longer extant. The specimen at G is chosen here as the lectotype.

When Rusby published *Bignonia tecomiflora*, he was able to base his description upon the complete sets of *Bang 1510* and *1596*. *Bang 1510* (NY, barcode 278056) is selected here as the lectotype based on its excellent condition and completeness: flowers, flower buds, capsules, and seeds are all present on this sheet. Gentry (1982) cited *Pyrostegia tecomiflora* and *B. tecomiflora* as synonyms under both *P. dichotoma* and *P. venusta*. The syntypes of *B. tecomiflora* have the inflorescences more crowded than is generally the case for *P. dichotoma*; however, the type of inflorescence trichomes, minute and appressed, is that generally found in *P. dichotoma* and not found in *P. venusta*. The staminode, in one flower dissected, was at the level of the stamen insertion and, in the other flower dissected, 3 mm above the level of insertion, better fitting the pattern of *P. dichotoma* than *P. venusta*, in which the staminodes are inserted (rarely 0.8)1.2–1.6 cm above the stamens.

The holotype of *Pyrostegia tubulosa* Bureau & K. Schum. (in Martius, Fl. Bras. 8(2): 231. 1897, *Richard Schomburgk 969*, from Kanuku, Guyana) is presumed to have been at B and destroyed. No isotypes were seen in this study or by Gentry (1982), and none are in the database of Schomburgk collections compiled by The Nationaal Herbarium Nederland (<http://www.nationaalherbarium.nl/>). However, three specimens of *Pyrostegia* made in the Kanuku Mountains of Guyana (*Jansen-Jacobs et al. 369*, *437*, and *Wilson-Browne 217*) were studied and not found to be different from other *P. dichotoma*. Bureau and Schumann (1897) used the margin of the calyx, undulate versus truncate, to help separate *P. tubulosa* from *P. dichotoma*. However, the calyx margin is very variable in *P. dichotoma*, and the margin exhibited by the collections from Guyana fit well into that range. Bureau and Schumann (1897) also used the nature of the leaf apex, rostrate versus acuminate to somewhat obtuse, to separate the taxa. The leaf apices of specimens examined from Guyana did not show a significant difference from those of other collections of *P. dichotoma*. The corolla described by Bureau and Schumann (1897), 6.8–7.3 cm long, and that of *Wilson-Browne 217*, 6.5–7 cm long, are larger than those otherwise seen. However, the dissected flower of *Jansen-Jacobs et al. 437* had a total corolla length of 6.2 cm, well within the range for *P. dichotoma*. Based on these observations, *P. tubulosa* is treated here as synonymous with *P. dichotoma* (following Gentry, 1982).

Pyrostegia dichotoma and *P. venusta* are very similar in general appearance. The most reliable way to separate the two is by the level of the staminode insertion. In *P. dichotoma*, the staminode is inserted with the stamens (Fig. 3F), and in *P. venusta*, it is inserted considerably (at least 8 mm) higher in the corolla tube (Fig. 3B). Bureau and Schumann (1897: fig. 98) and Lohmann and Pirani (1998: fig 10D) provide accurate and useful illustrations of the relative position of the stamens and staminode in *P. venusta*. *Pyrostegia dichotoma* almost always has the inflorescence trichomes minute and appressed to ascending, while in *P. venusta* the trichomes are perpendicular to the surface at least at the base (Fig. 3A). Fabris (1965: fig. 29) provides an excellent illustration of the trichome type found in *P. venusta*. However, it is not uncommon for individuals of both species to have nearly glabrous inflorescences, particularly after the bracteoles have been lost. Additionally, one specimen was seen, *Daly et al. 2271*, which had trichomes of the type generally found in *P. venusta*, but had all the other characteristics of *P. dichotoma*. *Pyrostegia dichotoma* tends to have a more open, often elongate, inflorescence that is 2 to 4 times branched (Fig. 3D; Gentry, 1982: fig. 38), while *P. venusta* generally has a very dense panicle, which is often subcorymbose and either unbranched or 1 or 2 times branched (Bureau & Schumann, 1897: fig. 98; Lohmann & Pirani, 1998: fig. 10A). However, some specimens of *P. venusta*, such as *Yoshiizumi s.n. (ESA-6363)* (from São Paulo, Brazil), *Harley 21606* (from Bahia, Brazil), and *Hassler 11784* (from Central, Paraguay), can have very open inflorescences, while some specimens of *P. dichotoma*, such as *Ducke 725* (from Amazonas, Brazil), *Mathias & Taylor 6080* (from Loreto, Peru), and *Gentry et al. 77514* (from Beni, Bolivia), can have fairly crowded ones. The two species do not appear to be sympatric. While *P. dichotoma* is known from Guyana, Suriname, Venezuela, Colombia, Peru, Bolivia, and Amazonian Brazil, *P. venusta* is native to Atlantic and southern Brazil, from Piauí to Rio Grande do Sul, southern Paraguay, and northeastern Argentina. The latter species is very widely cultivated and possibly naturalizes in some areas.

Selected specimens examined. BOLIVIA. **Beni:** Prov. Vaca Diez, E side of Riberalta, *J. C. Solomon 6148* (MO). **La Paz:** Sorata, *M. Bang 1596* (syntypes of *Bignonia tecomiflora*, A, BM, F, G, K not seen, M, MICH, MO, NY, S, W); Prov. Nor Yungas, Corocoro, 12 km NE of Caranavi, *A. H. Gentry et al. 44347* (MO). **Pando:** Prov. Manuripi, 30 km

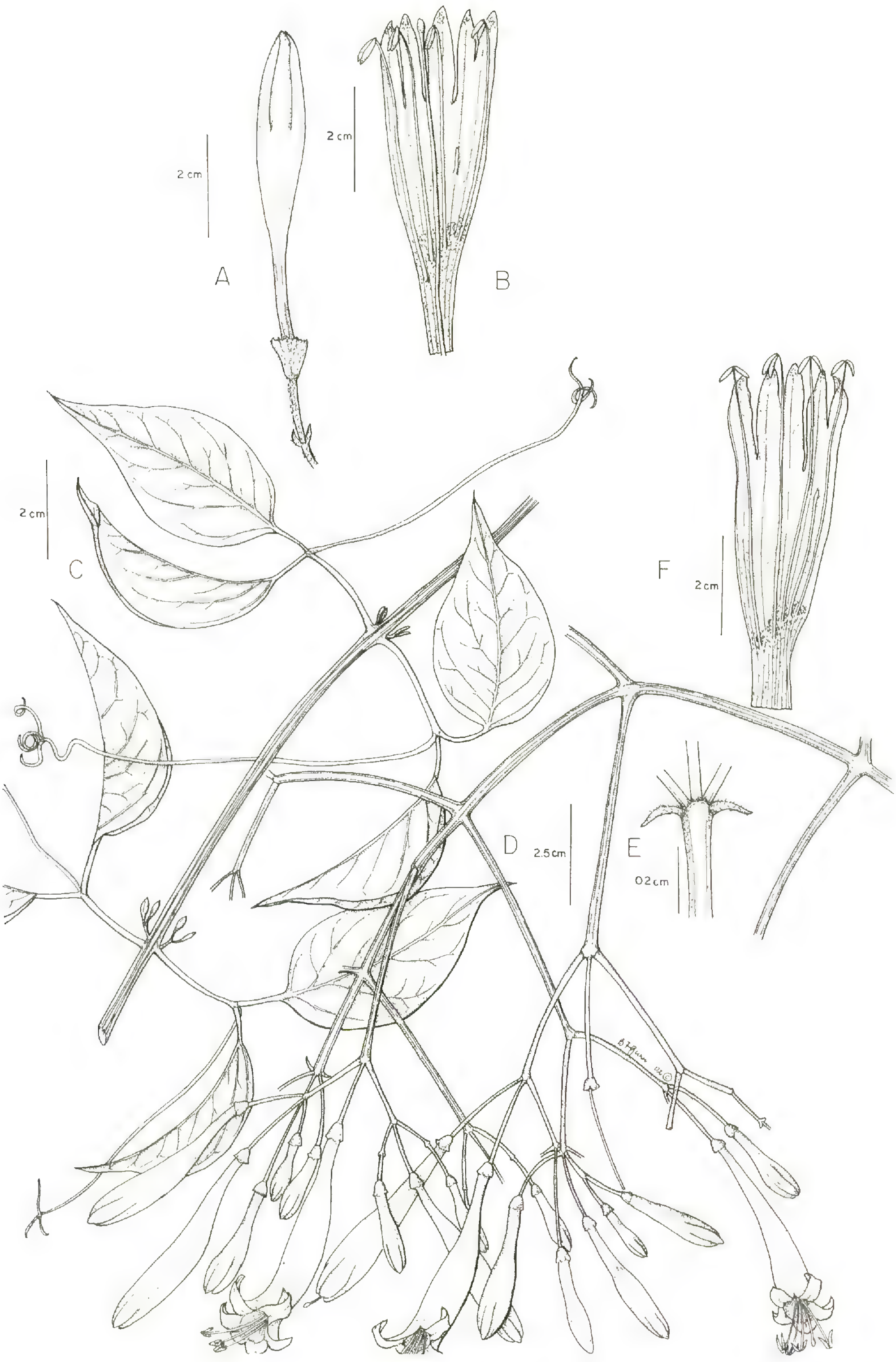

Figure 3. A, B. *Pyrostegia venusta* (Ker Gawl.) Miers (from *E. W. Schupp 258*, MO). —A. Flower bud. —B. Longitudinal section of corolla with androecium, style, and stigma. C–F. *Pyrostegia dichotoma* Miers ex K. Schum. —C. Branch with tendrillate leaves (from *B. Stergios et al. 8868*, MO). —D. Inflorescence. —E. Inflorescence node (D, E from *J. A. Steyermark et al. 109875*, MO). —F. Longitudinal section of corolla with androecium (from *S. F. Smith et al. 1051*, MO).

N de Puerto America, *A. Jardim et al. 2431* (MO). **Santa Cruz:** Prov. Velasco, Parq. Nac. Noel Kempff Mercado, Asseradero El Chore, *R. Guillén et al. 4108* (MO). BRAZIL. **Acre:** Mun. de Senador Guiomard, BR 317, Km 23, *C. A. Cid & B. Nelson 2821* (MO). **Amazonas:** Serra near Namorado Novo, watershed betw. Rio Curuquetê and Rio Madeira at Abunã, *G. T. Prance et al. 14705* (MO). **Mato Grosso:** BR 364, Cuiabá-Porto Velho, Distr. de Patronal, Vila Bela da Santíssima Trindade, *C. A. Cid et al. 4409* (MO). **Pará:** Near Embrapa station at Km 23 on rd. Altamira–Itaituba, *C. Berg et al. BG755* (MO). COLOMBIA. **Caquetá:** Km 90 on rd. from Neivo to Florencia, ca. 6 km NW of Florencia, *A. H. Gentry et al. 9067* (MO). **Meta:** Cordillera La Macarena, mesa del Río Sansa, *J. Idrobo et al. 1274* (MO). GUYANA. **Essequibo:** Kanuku Mtns., *M. J. Jansen-Jacobs et al. 437* (MO). PERU. **Loreto:** Pucallpa–Aguaytía rd., Km 37 W of Tournavista, *M. E. Mathias & D. Taylor 6080* (MO). **Madre de Dios:** Iberia, Río Tahuamanu, *R. J. Seibert 1952* (MO). **Pasco:** Prov. Oxapampa, Panjil, 12 km by air from Puerto Inca, *D. N. Smith & R. Foster 2416* (MO). **San Martín:** Prov. San Martín, Tarapoto, *F. Woytkowski 35089* (MO). SURINAME. **Nickerie:** Zuid River, Kayser airstrip, 45 km above confluence with Lucie River, *H. S. Irwin et al. 57636* (MO). VENEZUELA. **Amazonas:** Cerca del pueblo de Macuruco, *P. Berry 2123* (MO). **Bolívar:** Mun. Sucre, carr. desde Jabillal a Ciudad Bolívar, *E. Sanoja 2743* (MO). **Delta Amacuro:** Dept. Tucupita, betw. Los Castillos de Guyana and the town of Sierra Imataca, *G. Davidse & A. González 16437* (MO).

3. Pyrostegia millingtonioides Sandwith, Kew Bull. 15: 464. 1962. TYPE: Brazil. Pará: Obidos, in capoeira of terra firme, 23 July 1912, *A. Ducke s.n. (MG 12046)* (holotype, K not seen, K digitized image!; isotype, MG not seen).

Branchlets with dense to scattered curved-ascending trichomes, interpetiolar glandular fields absent, slight interpetiolar ridge present. Leaves 2-foliolate, often with an apically trifid terminal tendril; petioles 1.9–4 cm, with curving trichomes at least in adaxial canal; petiolules 0.5–5 cm; leaflets ovate, slightly subinequilateral, 4–11 × 2–6 cm, chartaceous, 3 to 5 pairs of lateral veins prominent abaxially, both surfaces densely to sparsely pilose to pilose only on veins or glabrous, pellucid-lepidote, often especially conspicuous abaxially, with large glands in the axils of lateral veins, base rounded or truncate, apex briefly acuminate-mucronulate, or acuminate-mucronulate. Panicle terminal, densely corymbose or paniculate with calyces overlapping in dried specimens, 2 to 4 times branched, peduncle, rachis, and bracteoles covered with minute, ascending-curved to appressed trichomes; calyx excluding denticules 3–4.5 × 5.5–6 mm at apex, with sparse lepidote scales, sometimes minutely puberulent, apex ciliate; corolla narrowly salverform, white; tube 4.1–5.5 cm long, 2–2.3 mm wide at base, ca. 3 mm wide at mouth, internally glabrous or pubescent near mouth, externally glabrous; lobes elliptic-oblong, 0.6–0.7 × ca. 0.4 cm, puberulent apically and marginally; stamens and staminode inserted at approximately same level in corolla tube, 3.9–4.4 cm from base of tube, stamen filaments 0.15–0.20 cm, thecae subparallel, 1.7–2 mm, staminode ca. 1 mm; disc 1.5–1.75 × ca. 1 mm; pistil ca. 4.4 cm, ovary 2–3 × ca. 0.8 mm, stigma lobes reniform-suborbicular. Fruit not known (Fig. 2A, B).

Distribution. Brazil, in the states of Pará and Maranhão; in dry secondary growth; elevation not recorded on labels (Fig. 1).

Phenology. Flowering in July and August.

Discussion. This species, with its strongly fragrant, white, salverform corollas, and stamens inserted near the mouth of the corolla tube, seems oddly placed in *Pyrostegia*, which otherwise has probably odorless (Gentry, 1990a), orange, narrow tubular-infundibular corollas with the stamens inserted below the middle of the corolla tube. *Pyrostegia cinerea*, *P. dichotoma*, and *P. venusta* are typical of hummingbird-pollinated Bignoniaceae flowers: odorless, the corolla bright red-orange (or deep red-violet), of fairly thick texture, with a narrow tube and wider mouth, and more or less glabrous internally (Gentry, 1980, 1990a), while the fragrant flowers of *P. millingtonioides*, with white, narrow, tubular, and thickly textured corollas, are probably moth pollinated (Gentry, 1980, 1990a). Gentry (1990a) reported that change of pollinator within a genus of Bignoniaceae is very rare and that only 18% of the Neotropical genera of Bignoniaceae have more than one type of pollen vector, and most of the species in these genera are overwhelmingly bee pollinated with one or two species switching to hummingbird pollination. Only two other genera of Neotropical Bignoniaceae, *Arrabidaea* and *Tabebuia* Gomes ex DC., have both moth- and hummingbird-pollinated species, and most of the species in those two genera are bee pollinated (Gentry, 1990a). *Pyrostegia* is the only genus reported (Gentry, 1990a) to have both hummingbird- and moth-pollinated species, with no species pollinated by bees.

On the other hand, there may be a positive correlation between dry habitats (such as that of *Pyrostegia millingtonioides*) and moth pollination. In a comparison of pollinators of lianas in different types of forests, Gentry (1991) suggested that a larger percentage of hummingbird-pollinated species is found in moist and wet forests (2%) or pluvial forests (7%) than in dry forests (1%), and a larger percentage of moth-pollinated species is found in dry forests (2%) versus moist and wet forests (0%) and pluvial forests (1%).

The insertion of the stamens above the middle of the corolla tube appears to be associated with the moth-pollinated syndrome. Gentry (1990a) listed three

genera in the Bignoniaceae as exclusively moth pollinated: *Tanaecium*, *Leucocalantha* Barb. Rodr., and *Sphingiphila* A. H. Gentry (listed as new genus). *Leucocalantha* (pers. obs.), *Tanaecium* (Macbride, 1961), and *Sphingiphila* (Gentry, 1990b) all have the stamens inserted above the middle of the corolla tube. Therefore, the main differences between *Pyrostegia millingtonioides* and *P. dichotoma* and *P. venusta* appear to be associated with pollen vector. The three species have many morphological characters in common: absence of interpetiolar glands, presence of generally 2-foliolate leaves with an apically trifid tendril, leaflets similar in shape and indument, calyx of similar shape, corolla tubes glabrous externally, corolla lobes more or less valvate at base in bud, stamens and stigma exserted, and a relatively wide stigma. In addition, as previously discussed, the pollen of all four species of *Pyrostegia* is 3(or 4)-collate and finely reticulate (Urban, 1916; Sandwith, 1962; Gentry & Tomb, 1979), and *P. millingtonioides*, *P. dichotoma*, and *P. cinerea* have similar wood anatomy (Santos, 1995). Alternative generic placement of *P. millingtonioides* is not suggested at this time.

Additional specimens examined. BRAZIL. **Maranhão:** Rodovia Açailândia–Santa Inês, Km 100, *G. S. Pinheiro 19* (MO); St. Luzia, Reserva Florestal de Buriticupu, propriedade da Floresta Rio Doce S.A. 52 km de Buriticupu, *G. dos Santos et al. 41* (MO), *42* (MO). **Pará:** Obidos, *A. Ducke s.n. (RB 16322)* (NY).

4. Pyrostegia venusta (Ker Gawl.) Miers, Proc. Roy. Hort. Soc. London 3: 188. 1863. Basionym: *Bignonia venusta* Ker Gawl., Bot. Reg. 3: tab. 249. 1818. *Tecoma venusta* (Ker Gawl.) Lem., Hort. Universel 5: 1. 1843. TYPE: tab. 249, illustration of greenhouse plant cultivated at Combe Wood, England, from seed originally from Rio de Janeiro, Brazil (lectotype, designated by Sandwith & Hunt, 1974: 75, tab. 249 in Ker Gawler, 1818!).

Bignonia ignea Vell., Fl. Flumin. 244. 1825 [1829]. *Pyrostegia ignea* (Vell.) C. Presl, Abh. Königl. Böhm. Ges. Wiss., ser. 5, 3: 523. 1845. TYPE: tab. 15 in Vellozo, Fl. Flumin. Icones 6. 1827 [1831] (lectotype, designated here, tab. 15 in Vellozo, 1827 [1831]!).

Pyrostegia venusta var. *villosa* Hassl. in Sprague, Bull. Herb. Boissier, sér. 2, 5: 84. 1905 [1904]. TYPE: Paraguay. Capibary, Dec., *E. Hassler 5940* (lectotype, designated here, G!; duplicates, A!, BM!, K not seen, MO!, NY!, S!, W!).

Branchlets glabrous, or with few trichomes at the nodes, or scattered to densely short-pilose to puberulent, interpetiolar glandular fields absent, interpetiolar ridge absent. Leaves 2-foliolate, often with an apically trifid terminal tendril (the ends rarely branched again, bifid or trifid), or leaves 3-foliolate; petioles 1–4 cm, densely pubescent, pilose in the adaxial canal or glabrous; petiolules 0.4–3 cm; leaflets ovate (rarely lanceolate), slightly subinequilateral, 2.5–11.5 × 1.2–6 cm, chartaceous (rarely membranous), 3 to 5 pairs of lateral veins prominent below, densely short-pilose to glabrous, pellucid-lepidote, often especially conspicuous abaxially, with large glands in the axils of lower lateral veins, base rounded or truncate (rarely cordate), apex briefly acuminate-mucronulate, or acuminate-mucronulate (obtuse-mucronulate or acuminate). Panicle usually terminal or axillary, generally dense or subcorymbose with calyces often overlapping in dried specimens, unbranched or 1 or 2(rarely 3) times branched, peduncle, rachis, and bracteoles nearly glabrous to densely puberulent or pilose, the trichomes initially perpendicular to the surface; calyx excluding denticules 4–8 × 4.5–10 mm at apex, with sparse lepidote scales, glabrous to densely short-pilose to puberulent, apex ciliate; corolla narrow tubular-infundibular, orange or reddish orange (rarely yellow); tube (rarely 2.7)4–7 cm long, 2–5 mm wide at base, 8–13 mm wide at mouth, internally sericeous at and below insertion of stamens and staminode, externally glabrous; lobes oblong, 1–1.8 × 0.3–0.7 cm, puberulent apically and marginally; stamens inserted 1.3–3.5 cm from base of corolla tube, staminode inserted (rarely 0.8)1.2–1.6 cm above insertion of higher stamens, stamen filaments (rarely 2.6)3.2–5.2 cm, thecae subparallel, (rarely 3)4–6.3 mm, staminode 1–8 mm (rarely developing an anther and then similar in length to, and inserted with, stamens); disc 1–3 × 2–3 mm; pistil 4.6–8.5 cm, ovary 4–6.5 × ca. 1 mm, stigma lobes broadly ovate, ovate, orbicular, or broadly oblong. Capsule 13.5–30 × 0.8–1.5 cm, glabrous, drying with olive cast, midvein apparent, but not conspicuous, base acute, apex aristate; seeds 0.7–1.4 × 2.8–4.5 cm (Fig. 3A, B; Fabris, 1965: fig. 29; Bureau & Schumann, 1897: fig. 98; Lohmann & Pirani, 1998: fig. 10A–E).

Distribution. Atlantic and southern Brazil, from Piauí to Rio Grande do Sul, southern Paraguay, and northeastern Argentina; 70–1300 m elevation, generally found below 1000 m. Frequently reported as growing in disturbed semi-evergreen forest or cerrado. Cultivated as an ornamental throughout the tropics and subtropics and possibly naturalizing in some areas (Fig. 1).

Phenology. Collected in flower in every month except December, peaking June to September. Fruiting July to December.

Common names. Brazil: Cipó de São João (Sandwith & Hunt, 1974; *Heringer 11982*), Cipó Caititu

(*Cutler 8404*), Cipó Tingá (*Carauta 408*), Dedo de Moça (*Emperaire & Campelo 2748*); Argentina: Pico de Tucán, Flor de San Juan (Fabris, 1965); Peru: Lluvia de Oro (*Seibert 2333*); Guatemala: Chiltote, Chorro de Oro, Chorro (Standley & Williams, 1974); El Salvador: San Carlos (Standley & Williams, 1974); Costa Rica: Triquitraque (Standley & Williams, 1974); U.S.A.: Flame Flower, Flaming-Trumpet, Golden-Shower (Bailey et al., 1976).

Discussion. The Vellozo plate 15 (Vellozo, 1827) was chosen as the lectotype of *Bignonia ignea*, as no Vellozo *Bignonia* specimens have been found (Gentry, 1975). Based on the Vellozo plate and description (Vellozo, 1825), *B. ignea* is placed in synonymy of *Pyrostegia venusta*, following Bureau and Schumann (1897), Sandwith and Hunt (1974), and Gentry (1982).

The six syntypes and 15 of the isosyntypes of *Pyrostegia venusta* var. *villosa* were seen, and *E. Hassler 5940* (G) is chosen as the lectotype, based on the quality of the specimen and its large number of widely distributed duplicates. Hassler (Sprague, 1905) distinguished *P. venusta* var. *villosa* from the typical variety based strictly on presence or absence of pubescence, the typical variety defined as glabrous to glabrescent and variety *villosa* as the whole plant more or less villous to pubescent. The type material of *P. venusta* var. *villosa* examined in this study had densely pubescent to pilose young branchlets, leaflet surfaces, and pedicels. In this study, specimens with similar pubescence were seen only south of Bahia, Brazil. Plants intermediate in pubescence, that is, with scattered trichomes on the pedicels but with the young branchlet internodes and the leaflet surface between the veins glabrous, were also restricted to this same geographical range. Nearly all specimens throughout the entire species range showed some degree of pubescence, with at least some trichomes present in the adaxial canals of the petioles and petiolules and on the bracteoles of the inflorescence. The trichomes are of the same type as those found on the more pubescent specimens. The less pubescent plants are found throughout the entire range of the species and, in fact, appear to grow in the same localities as the pubescent individuals. For example, the following pairs of pubescent and less pubescent plants were collected at the same localities and on the same dates: *Zardini 14343* and *Zardini 14365* and *Zardini 7193* and *Zardini 7131*. It seems more appropriate at this point not to formally recognize the variety.

Pyrostegia dichotoma has been treated as a synonym of *P. venusta* by some authors (Macbride, 1961). The two are treated here as separate but closely related species, as discussed below *P. dichotoma*.

Menninger et al. (1970) includes *Pyrostegia ignea* (= *P. venusta*) on his list of the most beautiful flowering climbers in the world, and ranks it as the most popular of all in the tropics. It is very widely cultivated and possibly naturalizes in some areas (Gentry, 1973). The specimen *Ferreira 192* indicated that the plant is considered toxic to cattle and has been used as a tonic and anti-diarrheic, but I have not seen a published account of this use. *Williams & Assis 6963* also recorded that the flowers are poisonous to cattle.

Selected specimens examined. ARGENTINA. **Corrientes:** Dep. Ituzaingó, Isla Apipé Grande, Panco-cué, *A. Schinini & R. Vanni 15813* (MO). BRAZIL. **Bahia:** Mun. de Mucuri, 14–17 km a W de Mucuri, *S. A. Mori et al. 10423* (MO); ca. 45 km N of Santa Maria de Vitória, on rd. to Serra Dourada, *R. M. Harley et al. 21606* (MO). **Ceará:** Serra de Araripe, near entrance to rd. to Novo Exu, *H. C. Cutler 8404* (MO). **Minas Gerais:** Mun. de Marliéria, Parque Estadual do Rio Doce, *E. P. Heringer 13984* (MO). **Paraíba:** Mun. de Maturéia, Pico do Jabre, Serra de Teixeira, *M. F. Agra 4398* (MO). **Paraná:** Faz. Água dos Indios (Cianorte), *G. Hatschbach 21599* (MO). **São Paulo:** Santa Genebra Forest Reserve, Barão Geraldo, near Campinas, *A. H. Gentry 59074* (MO). PARAGUAY. Atira, *E. Hassler 3022* (syntype of *Pyrostegia venusta* var. *villosa*, G; isosyntypes, A, BM, K not seen, NY, W), *3022-a* (syntype of *Pyrostegia venusta* var. *villosa*, G; isosyntypes, K not seen, NY); Chololo, *E. Hassler 6663* (syntype of *Pyrostegia venusta* var. *villosa*, G); Ipe-hu, *E. Hassler 5244* (syntype of *Pyrostegia venusta* var. *villosa*, G; isosyntypes, BM, NY, W); Concepcion, *E. Hassler 7363* (syntype of *Pyrostegia venusta* var. *villosa*, G; isosyntype, NY). **Caaguazu:** 103 km E of Coronel Oviedo on rd. to Puente Strossner, *A. H. Gentry 59440* (MO). **Guairá:** Cordillera de Ybytyruzú, 15 km N of Atena on rd. Melgarejo–Atena, *E. Zardini & R. Velásquez 13555* (MO). **Itapúa:** Pirapó, CEDEFO, *W. Hahn & L. Pérez de Molas 2760* (MO). **San Pedro:** 12 km al NE de Choré, *E. Zardini & C. Benítez 3401* (MO).

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Appendix 1. Index to Exsiccatae. Collections are alphabetical by collector name. After the collector's number, the number in parentheses corresponds to the species number in the List of Species. Type collections appear in boldface. All collections cited here were examined by this author as part of this study.

List of Species

1. *Pyrostegia cinerea* Bureau ex K. Schum.
2. *Pyrostegia dichotoma* Miers ex K. Schum.
3. *Pyrostegia millingtonioides* Sandwith
4. *Pyrostegia venusta* (Ker Gawl.) Miers.

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2911 (4). *Poloni et al. 1783* (4). *Prance 6277* (2). *Prance & Prance 21012* (1). *Prance & Schaller 26296* (4). *Prance et al. 755* (2), *5159* (2), *5410* (2), *5951* (2), *6642* (2), *7167* (2), *14705* (2), *59181* (2).

de Queiroz 1646 (4), *4722* (4). *Quevedo et al. 2479* (2). *Quirós 135* (4).

Raben 500 (4), *501* (4), *893* (4), *Raben s.n.* (4). *Rambo 29512* (4), *42050* (4), *42287* (4), *42403* (4), *42466* (4), *42569* (4), *42625* (4), *43028* (4), *47195* (4), *47286* (4), *48722* (4), *61199* (4). *Ratter et al. R1154* (2). *Rauscher 36* (4). *Read 1348* (4). *Reekmans 7326* (4). *Regnell 150* (4), *I-363* (4), *III-163* (4). *Reichardt 55* (4). *Reineck & Czermak 605* (4). *Reiss 82* (4). *Reitz 1174* (4), *1502-b* (4). *Reitz & Klein 2051* (4), *6846* (4), *9021* (4), *12642* (4), *12815* (4), *12855* (4), *13106* (4). *Renson 132* (4). *Renvoize et al. 3305* (4). *Reznicek et al. M38* (4). *Ribas et al. 2482* (4). *B. G. S. Ribeiro 1074* (2). *R. Ribeiro 278* (4). *Riedel s.n.* (2). *Ritter 33450* (4). *Roberston & Austin 1* (4). *de Rocha et al. 639* (4). *Rodrigues et al. 82* (4). *Rojas 1341* (4). *3221-A* (4). *Romieux s.n.* (4). *Rosa & dos Santos 1927* (2). *Rosales et al. 285* (2). *Rose et al. 14085* (4). *Rusby 1149* (2), *2725* (2). *Rutter 1851* (4).

Sagastegui A. 6880 (2). *Saldías et al. 2837* (2), *3014* (2). *Sampaio s.n.* (4). *Sanoja 2743* (2). *Santoro 415* (4), *587* (4). *E. dos Santos et al. 2673* (4). *G. dos Santos et al. 41* (3), *42* (3). *R. S. Santos 27993* (4). *R. S. Santos & Castellanos 24121* (1). *Sato s.n.* (1). *Saunders 803* (1). *Sazima 2622* (1). *Schala s.n.* (4). *Schallert 8118* (4). *Schwacke III-278* (1). *Schinini 6801* (4), *9105* (4), *13281* (4). *Schinini & Vanni 15813* (4). *Schinini et al. 11251* (4). *Schlieben 9979* (4). *Schomburgk 902* (2). *Schott 5970* (4). *Schuck s.n.* (4). *Schunke-V. 9795* (2). *Schupp 258* (4). *Schwarz 974* (4), *2727* (4), *2784* (4), *2927* (4), *2967* (4), *3088* (4), *3140* (4), *3191* (4), *3308* (4), *3363* (4), *4695* (4), *6077* (4). *Scolnik 1139* (2). *Segovia 28* (4). *A. Sehnem 3867* (4). *P. A. Sehnem 1627* (4). *S. J. Sehnem & P. A. Sehnem 3365* (4). *Seibert 1420* (4), *1927* (2), *1952* (2), *2109* (2), *2333* (4). *Seligson 690* (4). *Sello s.n.* (2), *Sello s.n.* (4). *Sendulsky 1009* (4). *Shepherd et al. 5817* (4). *Shuttleworth s.n.* (4). *Sick B871* (4). *D. S. Silva et al. 36* (4). *M. G. da Silva et al. 7108* (2). *Silvestre 61* (4). *Singh 204* (4). *D. N. Smith & Foster 2416* (2). *L. B. Smith & Reitz 5939* (4). *S. F. Smith et al. 1051* (2). *Solomon 6148* (2), *17076* (2), *18218* (4). *Soria 549* (4). *Soria et al. 1661* (4). *Soto Nunez & Martinez S. 6170* (4). *D. S. de Souza et al. 93* (4). *V. C. Souza & Ferraro 2593* (4). *Sperling & King 6416* (2). *Spruce* ***3930*** **(2)**. *Standley 5190* (4), *19243* (4), *19244* (4), *24629* (4), *39780* (1), *90933* (4). *Stergios 3236* (2). *Stergios et al. 8868* (2). *Steyermark 35373* (4). *Steyermark et al. 109875* (2), *124583* (2). *Stone 7414* (4). *Strang & Castellanos 1055* (4). *Streemadhavan 5716* (4). *Sturrock 86* (4). *Stutz 503* (4), *504* (4), *648* (4). *Sucre 7780* (4). *Sucre s.n. (RB 140961)* (4).

Tamashiro et al. 17 (4). *Tang 10351* (4). *Tavares 850* (4). *Taylor 17538* (4). *Teague 406* (4). *Teixeira et al. 1348* (2). *Tenerife 1* (4). *Tessmann 5402* (2). *Thomas 3988* (2). *Thorne & Ochoa 9702* (4). *Toro 108* (4), *593* (4). *Torres 1* (4). *Tscha et al. 312* (4). *von Tuerckheim 4012* (4).

Ule 8963 (2), *9781* (2).

J. Valerio R. 3089 (4). *J. Valerio Soto 26* (4). *Valverde 469* (4). *Vanni et al. 785* (4), *819* (4), *965* (4). *van der Veen s.n.* (4). *Venturi 3425* (4). *Vieira et al. 1051* (2). *Villalobos C. 35* (1).

Wagner 1142 (4). *Warming s.n.* (4). *Wasum s.n. (UCS 12137)* (4). *Wawra & Maly 486* (4). *Weddell 466* (4). *Weinberg 546* (4). *Weir 8* (4). *van der Werff 8243* (4). *West 140* (4). *Widgren 237* (4). *Widgren s.n.* (4). *L. Williams 11277* (2). *L. H. J. Williams 4* (4). *L. O. Williams & Assis 6963* (4), *7495* (4). *R. S. Williams 755* (2), *879* (2), *1574* (2). *Wilson-Browne 217* (2). *Windisch 632* (2). *Woolston 530* (4). *Wortley 1847* (4). *Wovtkowski 35089* (2). *Wurdack & Wright 406* (2).

Yena 257 (2).

Yanagizawa s.n. (SP 8988) (4). *Yoshiizumi s.n. (ESA-6363)* (4). *Yuncker 17917* (4). *Yuncker et al. 6092* (4).

Zanoni & Mejía 11909 (4). *Zardini 4410* (4), *4512* (4), *5023* (4), *5423* (4), *5569* (4), *5898* (4), *6445* (4), *6678* (4), *6746* (4), *6777* (4), *7113* (4), *7131* (4), *7193* (4), *7213* (4), *7274* (4), *13724* (4), *14343* (4), *14365* (4), *14728* (4). *Zardini & Aguayo 14394* (4), *14846* (4), *14913* (4). *Zardini & Benítez 3358* (4), *3401* (4). *Zardini & Ortiz 3070* (4). *Zardini & C. Velásquez 12504* (4), *12547* (4), *13027* (4), *13857* (4), *13896* (4), *14266* (4), *14282* (4), *14287* (4), *21703* (4). *Zardini & R. Velázquez 12415* (4), *13212* (4), *13223* (4), *13555* (4), *13608* (4), *13660* (4), *14223* (4), *14498* (4), *14501* (4), *14552* (4), *14608* (4). *Zardini & S. Velázquez 13345* (4), *13761* (4), *16271* (4). *Zardini & V. Velázquez 15197* (4). *Zardini et al. 12238* (4). *Zika 2508* (4). *Zuloaga et al. 2122* (4).

ANATOMY AND HISTOCHEMICAL LOCALIZATION OF LIPID SECRETIONS IN BRAZILIAN SPECIES OF *PANICUM* SECT. *LOREA* (POACEAE, PANICOIDEAE, PANICEAE)[1]

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ABSTRACT

This paper constitutes the first report of the chemical nature, and histochemical localization of secretions in Brazilian species of *Panicum* L. sect. *Lorea* Zuloaga (Poaceae, Panicoideae, Paniceae); the study also includes an anatomical analysis. Lipid secretions were found in leaf sheaths and ligular regions. Lipids accumulate in specialized epidermal cells, at times surrounding the bases of the macrohairs, and in the mesophyll. Viscous secretions have been previously reported for *P. vaginiviscosum* Renvoize & Zuloaga and *P. acicularifolium* Renvoize & Zuloaga, species in section *Lorea* that are analyzed anatomically here. Lipid secretions are now also reported for the first time in eight other species of section *Lorea*: *P. bahiense* Renvoize, *P. chnoodes* Trin., *P. cipoense* Renvoize & Send., *P. durifolium* Renvoize & Zuloaga, *P. euprepes* Renvoize, *P. molinioides* Trin., *P. poliophyllum* Renvoize & Zuloaga, and *P. trinii* Kunth.

Key words: Gramineae, herbivory, lipids, *Panicum*, plant defenses, Poaceae, secreting epidermal cells.

The genus *Panicum* L., s.l., includes nearly 400 species and exhibits a worldwide distribution (Clayton & Renvoize, 1986). As presently circumscribed in its strict sense (Aliscioni et al., 2003), the genus should be limited to only subgenus *Panicum*, with five sections and approximately 100 species worldwide and 68 taxa in the Americas. The other previously considered subgenera *Dichanthelium* Hitchc. & Chase, *Megathyrsus* Pilg., *Phanopyrum* (Raf.) Pilg., and *Steinchisma* Raf. have been raised to the generic level or their species have been transferred to other genera (Zuloaga et al., 1998; Simon & Jacobs, 2003; Aliscioni et al., 2003). Also, according to Aliscioni et al. (2003), there are several species or sections of dubious taxonomic position within the Paniceae, i.e., species previously grouped in subgenus *Agrostoides* (Hitchc. & Chase ex Hsu) Zuloaga and *Phanopyrum*. Species of section *Lorea* Zuloaga (included by Zuloaga [1987] in subgenus *Phanopyrum*) are currently considered among these taxa declared incertae sedis (Aliscioni et al., 2003). As presently circumscribed, section *Lorea* includes 27 species, of which five are endemic to Venezuela and the Guayana Highlands; also, 21 species are restricted to central and southern Brazil. Here they inhabit the highlands of Bahia and Minas Gerais, the cerrados (s.l.) of central Brazil to coastal regions of Rio de Janeiro and Bahia, where a few species occur in specialized cerrados, known as restingas, such as *Panicum restingae* Renvoize & Zuloaga, *P. sacciolepoides* Renvoize & Zuloaga, and *P. marauense* Renvoize & Zuloaga (Zuloaga, 1987; Renvoize & Zuloaga, 1995). *Panicum chnoodes* Trin. is the only species known to be disjunct between the Guayana Highlands and central Brazil.

Panicum sect. *Lorea* is distinguished by its tussock habit, pungent leaf blades, and usually indistinct junction of sheath and blade (Renvoize, 1978; Renvoize & Zuloaga, 1984). Plants are caespitose, perennial, with basal persistent sheaths, with their blades lanceolate or linear-lanceolate, either pilose or glabrous (Renvoize & Zuloaga, 1984). The presence of viscose elements has been mentioned previously to occur in the sheaths and around the ligular region in only two species of this section, *P. vaginiviscosum* Renvoize & Zuloaga and *P. acicularifolium* Renvoize & Zuloaga (Renvoize & Zuloaga, 1995). Until now, no anatomical or histochem-

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ical studies have been made on any species of this group. We describe the anatomy of the secreting epidermal cells, and the lipid nature of their secretions is histochemically confirmed. Lipid secretions are anatomically confirmed for the first time in 10 species of *Panicum* sect. *Lorea*: *P. acicularifolium*, *P. bahiense* Renvoize, *P. chnoodes*, *P. cipoense* Renvoize & Send., *P. durifolium* Renvoize & Zuloaga, *P. euprepes* Renvoize, *P. molinioides* Trin., *P. poliophyllum* Renvoize & Zuloaga, *P. trinii* Kunth, and *P. vaginiviscosum*.

Essential oils in the Poaceae have been previously reported from roots, stems, leaves, or inflorescences of species of *Anthoxanthum* L. (= *Hierochloe* R. Br.), *Bothriochloa* Kuntze, *Chrysopogon* Trin. (= *Vetiveria* Bory), *Cymbopogon* Spreng., *Elionurus* Humb. & Bonpl. ex Willd., and *Melica* L. (Guenther, 1950; Arber, 1965; Pinder & Kerr, 1980; Watson & Dallwitz, 1992; Kaul & Vats, 1998). Most of these aromatic grasses follow the C_4 metabolic pathway of carbon fixation, and approximately 88% are members of the Andropogoneae, a tribe that spread globally by the late Miocene, possibly the period of greatest evolutionary diversity for aromatic grasses (Kaul & Vats, 1998). When ingested, these compounds slow the growth of herbivores, making them more vulnerable to predators and parasitoids (Coley & Barone, 1996), constituting a selective agent for plant defense against herbivory. Also, Ellis (1990) reported the presence of tannin-like substances in 104 species of Poaceae, with many representatives in the Andropogoneae and Arundinelleae, and occasionally in a few members of the Paniceae (two species of *Digitaria* Haller, three species of *Echinochloa* P. Beauv., and one species of *Panicum*: *P. coloratum* L.). Ellis (1990) mentioned that, chemically, tannins are a heterogeneous group of phenol derivatives.

Previous studies carried out in the Poaceae showed that secretory substances are produced and accumulated in microhairs (Amarasinghe & Watson, 1988), in the single cells adjacent to photosynthetic and nonphotosynthetic tissues and between vascular bundles (Lewinsohn et al., 1998), in elaiosomes (Bresinsky, 1963; Berg, 1985; Davidse, 1987; Morrone et al., 2000), or by four different types of multicellular glands (Linder et al., 1990). The first type of multicellular gland is composed of epidermal cells forming a pad of cushion-based macrohairs (Bowden, 1971; Ellis, 1979); the second type is formed by numerous basal cells constituting the gland but not associated with macrohairs (Nicora, 1941; Davidse, 1988; Linder et al., 1990); the third type is comprised of multicellular glandular hairs (Kabuye & Wood, 1969); and the fourth type occurs as multicellular stalked glands with a central depression (Davidse, 1988; Zuloaga & Sendulsky, 1988).

Two types of glandular macrohairs were reported in *Panicum* (Kabuye & Wood, 1969): unicellular with swollen tips, present in *P. deustum* Thunb., and multicellular clavate glandular hairs, present in six species of tropical East Africa. Both types contain a yellowish and sticky substance at maturity. Multicellular glands were also reported on the lower lemma in species of *Panicum* sect. *Stolonifera* Hitchc. & Chase ex Pilg. (Zuloaga & Sendulsky, 1988), but secretory activity was not demonstrated.

The objective of this paper is to determine the nature and histochemical localization of the secretion and to describe the secretory epidermal cells in Brazilian species of *Panicum* sect. *Lorea*.

Materials and Methods

Plant Materials

Herbarium specimens of 16 species of *Panicum* sect. *Lorea* were studied in order to detect specialized secreting epidermal cells (see Appendix 1).

Anatomical Studies

Sheaths and blades were fixed in FAA (1 formalin: 4 alcohol 96%: 1 acetic acid) for 48 hours, and then stored in 70% ethanol, or they were removed from herbarium specimens and rehydrated in 5% Contrad 70 (Decon Laboratories, King of Prussia, Pennsylvania, U.S.A.) (Schmid & Turner, 1977) over 24 hours at 20°C. The material was desilicificated in hydrofluoric acid (5%) for 24 hours. Then the material was washed in distilled water, dehydrated in an ethanol series, and embedded in paraffin. Transverse and longitudinal sections 10 µm thick were cut on a rotary microtome. Sections were double stained with safranin-fast green.

Preliminary observations of the leaf material stained with Sudan IV (Gahan, 1984) showed that they are rich in lipids. Stained transversal sections of the leaf sheath were analyzed to detect and determine the localization of lipid secretions in the different tissues. We used only herbarium material for this study.

Light microscope (LM) studies were made using a ZEISS Phomi III microscope (Zeiss, Oberkochen, Germany), with a 35 mm photographic camera and Kodak (Rochester, New York, U.S.A.) Gold ASA100 film.

For observation with SEM, pieces of the middle portion of the leaf sheaths were mounted on stubs, carbon coated in a vacuum evaporator, and coated with a palladium alloy. The observations were made using a ZEISS DSM 940 A microscope at the Instituto de Botánica Darwinion, Argentina. Complementary observations of secretory structures were made with an

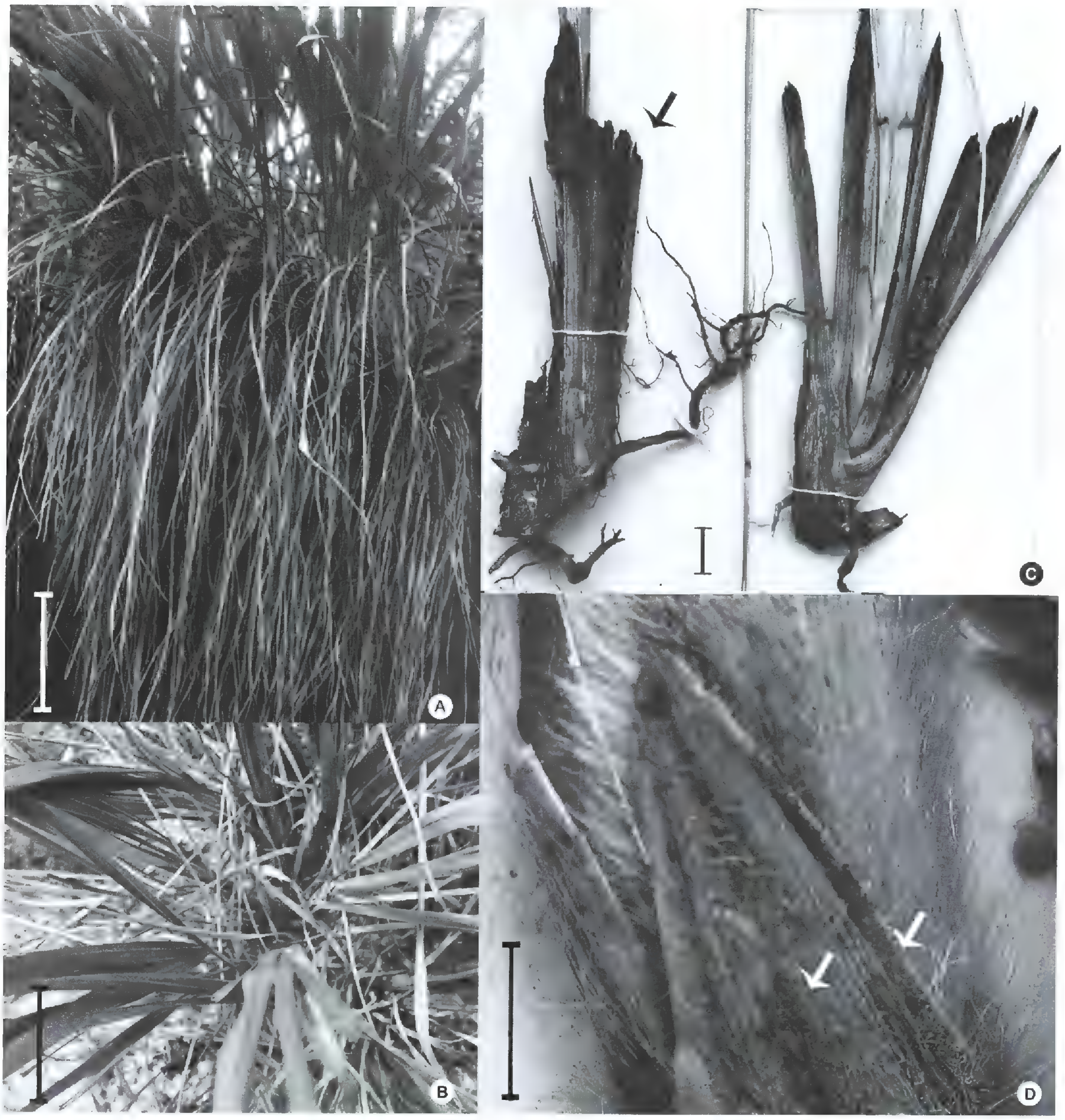

Figure 1. —A. *Panicum poliophyllum* growing in Caraça, Minas Gerais, Brazil. —B. Base of the plant of *P. poliophyllum*, showing dry leaf sheaths and young innovations. —C. Herbarium specimen of *P. subtiramulosum* (Caraça, Minas Gerais, Brazil: *Fonseca & Alvarenga 2109*, SI) showing evidences of fire (arrow). —D. Herbarium specimen of *P. bahiense* from Bahia, Brazil (*Zuloaga et al. 4850*, SI), with secretory structures (arrows) in the cataphyll. Scale bars: A, B = 10 cm; C = 1 cm; D = 0.5 cm.

environmental SEM in an XL30 ESEM (Phillips, Eindhoven, The Netherlands) at the Instituto Nacional de Tecnología Industrial (INTI), Argentina.

Results

Field observations

Plants of *Panicum* sect. *Lorea* collected from Brazil grow in campos rupestres, rocky grasslands, at 1070–1600 m. In all of the examined material, the distal culms, covered with young leaves, are protected by abundant and dry basal sheaths, constituting an important source of combustible material during fires in the cerrado landscape (Fig. 1A, B). In some herbarium specimens, burned leaf remains can be readily observed, and burning is a regular phenomenon in cerrado environments in central Brazil. When these grasses are burned, young culms are protected at their base by cataphylls and remnants of basal burned sheaths (Fig. 1C, D).

Field observations of some species of *Panicum* sect. *Lorea* showed the presence of large amounts of sticky secretions, with a strong and disagreeable smell.

Table 1. Localization of lipid secretions for 10 of the 16 species investigated in *Panicum* sect. *Lorea* with Sudan IV in transverse sections of leaf sheath. + = present, − = absent.

Species	Sheath indumentum	Mesophyll	Epidermis		Specialized secreting structures
			Abaxial	Adaxial	
P. aciculariforme	villous	+	+	+	
P. bahiense	villous	+	+	++	+ (unicellular)
P. chnoodes	villous	+	+	++	
P. cipoense	villous	+	+	−	−
P. durifolium	villous	+	+	++	−
P. euprepes	subglabrous	+	+	+	+ (unicellular)
P. molinioides	villous	+	++	+	+ (multicellular)
P. poliophyllum	subglabrous	+	+	−	+ (unicellular)
P. trinii	villous	+	+	++	
P. vaginiviscosum	subglabrous	+	+	++	

These secretions are restricted to leaf sheaths and ligular regions, which turn viscid. Usually, secretions are more abundant in cataphylls (Fig. 1D) and basal sheaths while young plants are growing, but they are almost absent in upper leaf sheaths. Basal sheaths are strongly attached to the culms, and marginal hairs are impregnated with sticky secretions that enhance the adhesion of sheaths to the culms.

ANATOMICAL STUDIES

Basal sheaths are villous all over or pilose on the margins and subglabrous on the surface (Table 1). Species in *Panicum* sect. *Lorea* with villous basal sheaths, e.g., *P. aciculariforme*, *P. bahiense*, *P. chnoodes*, *P. cipoense*, *P. durifolium*, *P. molinioides*, and *P. trinii*, show conspicuous costal and intercostal zones (Fig. 2A). Macrohairs are restricted to intercostal areas (Fig. 2A–C) and are unicellular, stiff, with slightly thickened walls, and their bases are sunken and surrounded by a group of secreting epidermal cells slightly raised above the general level of the sheath surface (Fig. 2C). This type of macrohair is known as a cushion hair (Metcalfe, 1960; Ellis, 1979).

Species in *Panicum* sect. *Lorea* with subglabrous basal sheaths as in *P. euprepes*, *P. poliophyllum*, and *P. vaginiviscosum* show few undulations associated with vascular bundles. Solitary or grouped secreting

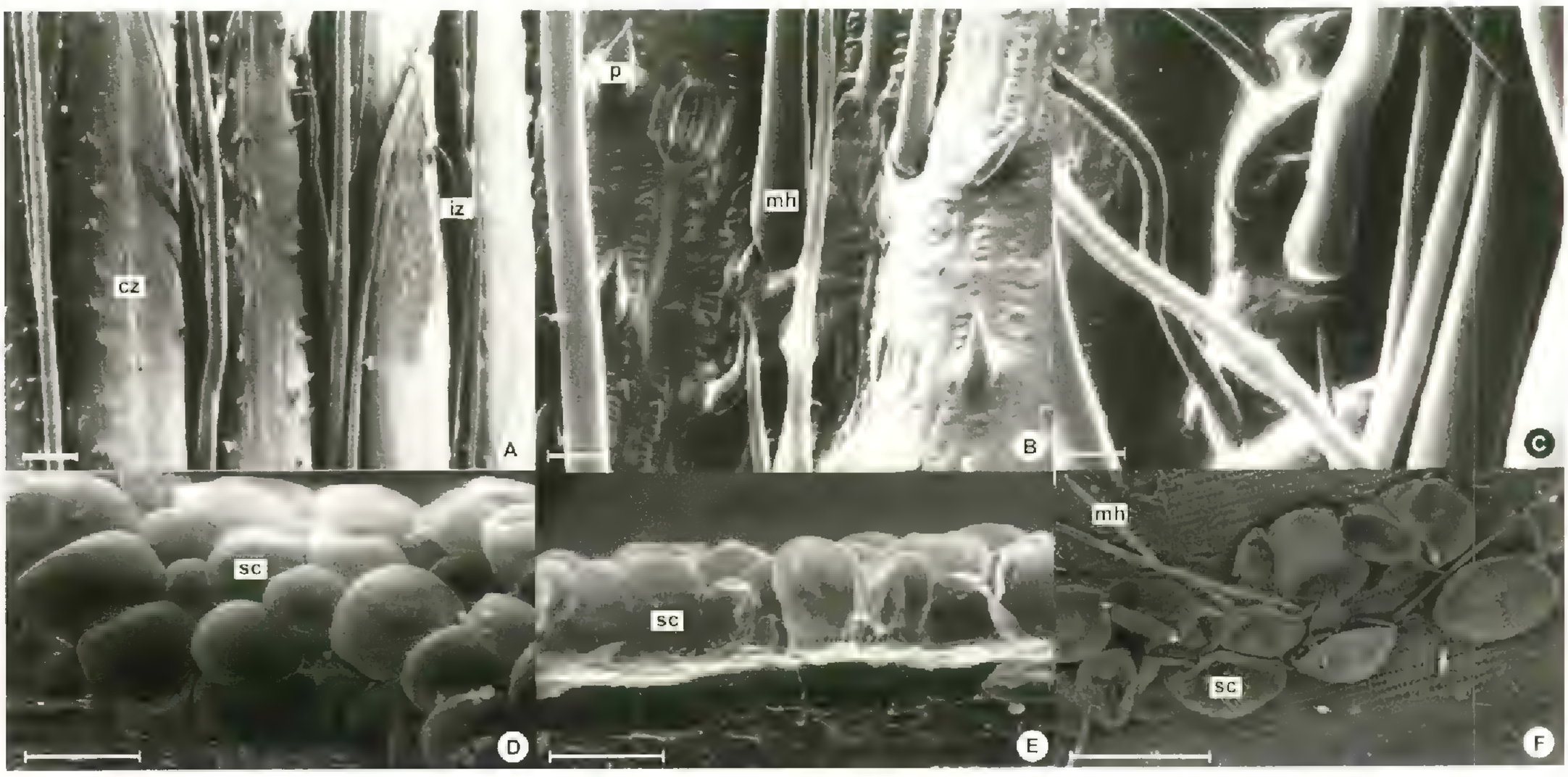

Figure 2. SEM microphotographs of the leaf sheath in *Panicum* sect. *Lorea*. —A. General view of the abaxial surface showing the position of macrohairs at intercostal zones. —B. Macrohairs with specialized epidermal cells in young leaf sheaths. —C. Macrohairs with collapsed, specialized epidermal cells after secretion in mature leaf sheaths. —D, E. Groups of specialized epidermal cells. —F. Groups of specialized epidermal cells after secretion. A, B, D–F: *P. euprepes* (*Zuloaga et al. 4783*, SI); C: *P. vaginiviscosum* (*Zuloaga et al. 4781*, SI). cz, costal zone; iz, intercostal zone; mh, macrohairs; p, prickle; sc, specialized epidermal cells. Scale bars: A–C = 20 μm; D, E = 100 μm; F = 200 μm.

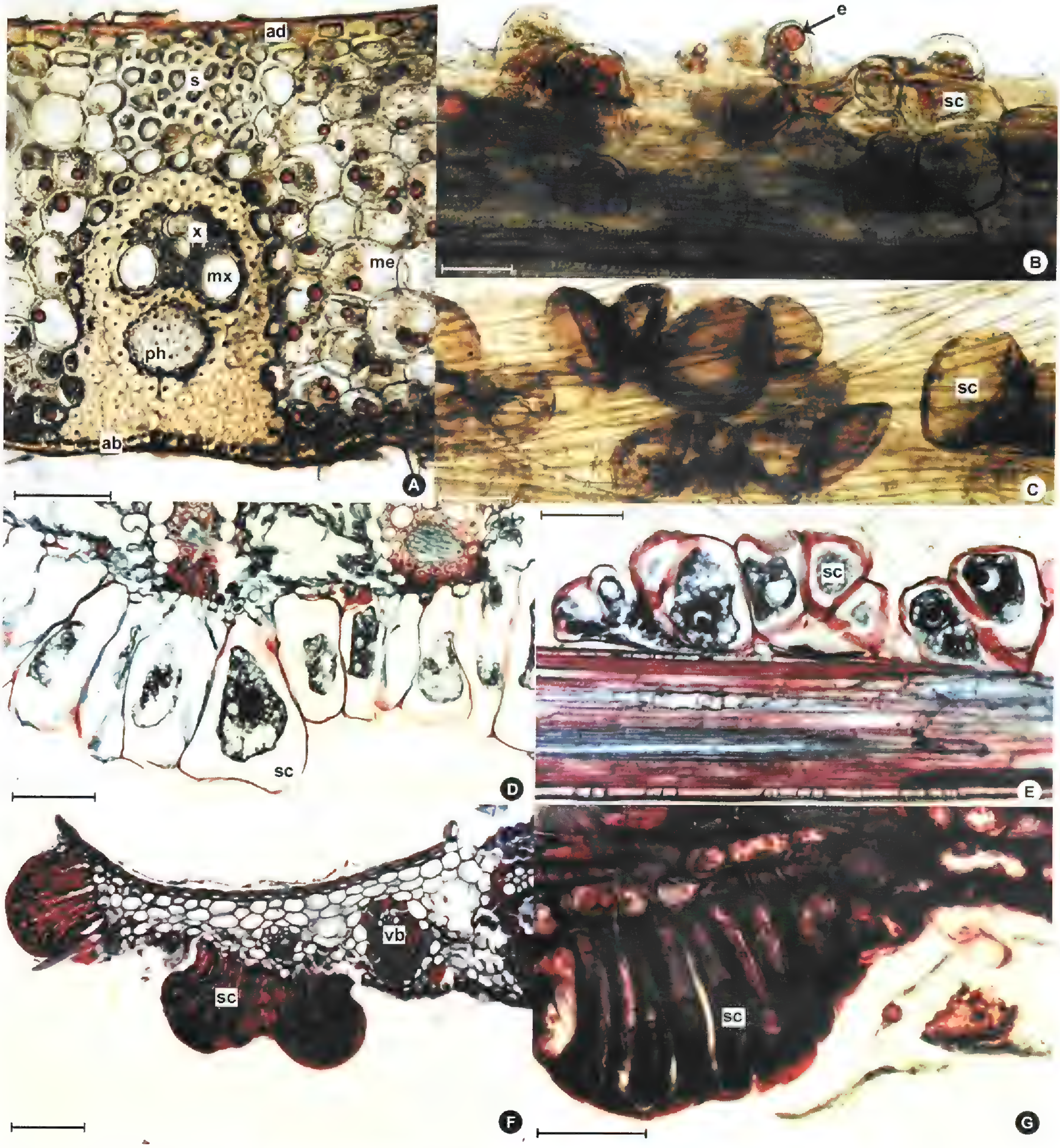

Figure 3. LM photographs of the leaf sheath in *Panicum* sect. *Lorea*. —A. Transverse section stained with Sudan IV. —B. Active cells stained with Sudan IV. Note elaioplasts at arrow. —C. Inactive and lignified cells, staining negatively to Sudan IV. D. E. Active cells stained with safranin-fast green. —D. Transverse section. —E. Longitudinal section. —F. Multicellular-secreting structures in leaf sheath transverse section. —G. Detail of a multicellular-secreting structure in leaf sheath transverse section. A: *P. vaginiviscosum* (*Zuloaga et al. 4781*, SI); B–E: *P. euprepes* (*Zuloaga et al. 4783*, SI); F–G: *P. molinioides* (*Zuloaga & Morrone 4701*, SI). ab, abaxial epidermis; ad, adaxial epidermis; e, elaioplast; me, mesophyll; mx, metaxylem; ph, phloem; s, sclerenchyma; sc, specialized epidermal cells; vb, vascular bundles; x, xylem. Scale bars: A–F = 100 μm; G = 50 μm.

specialized epidermal cells are present in both surfaces, although they are more frequent on the abaxial surface and often surround the bases of the macrohairs (Fig. 2D–F). These cells are not so abundant, although secretions are detected in fresh and herbarium material.

All species examined in transverse section show a unistratified epidermis composed of quadrangular to tabular cells (Fig. 3). On both surfaces, the epidermal cells have a thick outer tangential cell wall. The chlorenchyma has isodiametric to irregularly shaped cells. In viscid sheaths, only mesophyll cells contain abundant elaioplasts with lipids. The sclerenchyma is subepidermic and discontinuous, and forms adaxial and abaxial girders associated with primary and secondary vascular bundles. Fibers are lignified.

Transverse sections of the leaf sheaths observed from herbarium material of *Panicum* sect. *Lorea* show a positive reaction to Sudan IV. Lipids are accumulated in the mesophyll and also in the xylem and phloem parenchyma of the vascular bundles (Fig. 3A). Lipids are especially abundant inside the specialized epidermal cells (Fig. 3B). However, we did not observe specialized pores through which lipids are secreted. Specialized epidermal cells, once transformed in inactive cells, are persistent on the sheath surface and easily detected under magnification by their lignified and brownish-colored walls (Fig. 3C).

In transverse and longitudinal sections of the leaf sheaths, specialized secretory epidermal cells are globose to claviform in shape, according to their density on the surface (Fig. 3D, E). Their walls are thin and lignified, apparently not interrupted by specialized pores. The epidermal origin of these secreting cells is clearly demonstrated. The cytoplasm of these cells is dense, with abundant vesicles of different sizes. The nucleus is prominent and of variable placement with one or two visible nucleoli (Fig. 3D, E).

The presence of lipids was detected in 10 species: *Panicum acicularifolium*, *P. bahiense*, *P. chnoodes*, *P. cipoense*, *P. durifolium*, *P. euprepes*, *P. molinioides*, *P. poliophyllum*, *P. trinii*, and *P. vaginiviscosum* but not in the remaining six analyzed in section *Lorea*: *P. animarum* Renvoize, *P. lagostachyum* Renvoize & Zuloaga, *P. loreum* Trin., *P. lutzii* Swallen, *P. restingae*, and *P. subtiramulosum* Renvoize & Zuloaga. Lipids were detected in mesophyll and both epidermides with different accumulations in the adaxial and abaxial surfaces according to the species (Table 1). Lipids appear abundant on adaxial epidermis of *P. bahiense*, *P. durifolium*, *P. trinii*, and *P. vaginiviscosum*. The presence of unicellular specialized epidermal cells was observed in *P. bahiense*, *P. euprepes*, and *P. poliophyllum*. In *P. bahiense*, specialized secretory cells are distributed in intercostal zones and are associated with macrohairs. *Panicum molinioides* possesses multicellular secretory structures distributed in abaxial costal and intercostal zones that are generally not associated with macrohairs. In *P. molinioides*, glands are composed of a group of cells with a slightly concave central area. In transverse sections, these cells are axially elongated and are all surrounded by a distinct and thick cuticle (Fig. 3F, G).

Discussion

Fire has occurred in tropical savannas for thousands of years, shaping the landscape and selecting for adapted flora and fauna (Ramos Neto & Pivello, 2000). Many studies have concentrated on how plant species adapt to fire in the Brazilian cerrados (Coutinho, 1990). However, the herbaceous layer of the cerrado has not been studied as intensively as the woody layer (Mistry, 1998), and more knowledge is needed about the mechanism put in place in native herbaceous plants to survive herbivory after fire.

The tropical humid climate of the cerrado region has a dry winter when grasses dry out, and a wet summer. The burning season occurs from May to September, when the herbaceous vegetation is dry and more flammable. In the early wet season (September–October), fire occurrences decrease, although the vegetation is still capable of maintaining a fire (Ramos Neto & Pivello, 2000). Soils under cerrado vegetation are generally very poor in mineral nutrients, with toxic levels of aluminum and high acidity (Coutinho, 1982). After fires, the ash is highly beneficial to the growth of herbaceous and undershrub plants with superficial root systems, since they are provided with a large quantity of mineral nutrients and a significant reduction in aluminum toxicity (Coutinho, 1990). The ground layer plants are xeromorphic, usually with rather stiff leaves with smooth or harsh surfaces, although some grasses and forbs have soft, hairy leaves (Eiten, 1982).

Plants growing in a cerrado sensu stricto (with tree and scrub forms, where trees do not form a continuous canopy, Eiten, 1982) are mostly perennials, have a gray and dusty appearance, and possess hard siliceous leaves, sometimes even densely hairy, and with underground organs well adapted to burning. Furthermore, in species of *Panicum* sect. *Lorea*, basal sheaths are strongly attached to the culms, and marginal hairs are impregnated with sticky secretions, enhancing the adhesion of sheaths to the culms. These characteristics may represent a strategy to decrease the entrance of insects inside the leaf sheath, and perhaps also to reduce dehydration and combustibility. Fresh green leaves are produced shortly after burning; intense flowering can also be observed a few days or weeks after cerrado fires (Coutinho, 1982; Silberbauer-Gottsberger, 1984; Miranda et al., 2002). According to Marquis et al. (2002), a peak in herbivore abundance is associated with the flush of new leaves during the initial part of the wet season. Furthermore, cerrado plants most commonly attacked by insects can evade these herbivores by evolving chemically novel toxins as deterrents to distinguish themselves from their neighbors (Marquis et al., 2002). Ellis (1990) postulated that the presence of tannin-like substances (TLS) in species of grasses is primarily associated with sour grasslands and savannas of southern Africa, and that this feature may be related to a chemical defense mechanism as a response to damage caused by herbivores.

In species of *Panicum* sect. *Lorea*, the production of lipids, combined with a repellent substance, might well be associated with the sprouting of fresh green leaves, making the foliage unpalatable for grazing animals or predators. That association would account for the occurrence of secreting cells only on the cataphylls, leaf sheaths, and ligular regions of basal foliage, but not in leaves of the upper culms. In 10 of the 16 analyzed species of section *Lorea*, of a total of 27 species included in this section, lipid secretions are concentrated in epidermal cells, especially at the bases of the macrohairs, and in mesophyll. Globose-secreting cells are not so abundant, although secretions were detected in fresh material during fieldwork and in herbarium material. Although the presence of sticky substances and specialized secreting epidermal cells was not detected in three of the studied species of this section, the vast majority contained both and are likely to occur in other unstudied taxa of section *Lorea*. In general, species of this section occur in areas seldom visited by botanists, making collections scarce (Renvoize, 1978), but it is important to collect them with the complete basal portion to locate secretory structures or substances.

We have shown that the analyzed species of *Panicum* sect. *Lorea* have important differences in the presence and type of secretory structures. Lipids are present in the epidermis and mesophyll in 10 of the 16 species studied. Specialized cells are present or absent and, when present, are unicellular or grouped in multicellular structures.

Little information has been published in relation to interactions among other genera of lipid-secreting grasses and herbivores. In some species of the genus *Pentaschistis* (Nees) Spach inhabiting southern Africa and Madagascar, shoots are aromatic (or fetid) due to the presence of multicellular glands at the base of the macrohairs on the abaxial leaf blade surface, sheaths, pedicels, or glumes (Linder et al., 1990; Watson & Dallwitz, 1992). Plants, such as species of *Prionanthium* Desv., that are sticky and/or produce an unpleasant smell originating from an unknown volatile substance, are postulated to have an anti-herbivore mechanism (Davidse, 1988). Furthermore, a similar type of macrohair to that described in some species of *Panicum* sect. *Lorea* was reported in *Andropogon gayanus* Kunth var. *bisquamulatus* (Hochst.) Hack. (Andropogoneae) by Bowden (1971), but this author interpreted that the epidermal cells that surround macrohair bases are nectaries.

Most of the aromatic grasses studied up to the present are Andropogoneae and follow the C_4 pathway of carbon fixation (Kaul & Vats, 1998). The genus *Panicum*, s.l., includes all photosynthetic types: C_3, C_4 (NAD malic enzyme [NAD-ME], phosphoenolpyruvate carboxykinase [PCK], or NADP malic enzyme [NADP-ME]), and also C_3/C_4 intermediate species. This analysis shows that many species of *Panicum* sect. *Lorea*, which includes only C_3 species, have essential oils. Aliscioni et al. (2003) have suggested that *Panicum* sect. *Lorea* should be segregated from *Panicum*, because the species of this section that were studied were placed in an independent and strongly supported clade separate from *Panicum* s. str. As previously mentioned, species of this section share a similar habitat and distribution, as well as a suite of morphological characters. The presence of secretory tissue in species of this section may represent another unique character for this group. Nevertheless, the presence of secretory tissues should be analyzed in more species of *Lorea* and in new material of those species where glands were not found in this study, to confirm if this character represents a distinguishing feature for the group.

Addendum

While this paper was in press, a new contribution (Sede et al., 2008) presented a phylogenetic analysis of section *Lorea*, which led to the segregation of two new genera in the Paniceae. Species discussed here were rearranged in both *Apochloa* Zuloaga & Morrone and *Renvoizea* Zuloaga & Morrone.

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APPENDIX 1. Herbarium specimens of *Panicum* sect. *Lorea* species studied to detect specialized secreting epidermal cells.

P. aciculariifolium Renvoize & Zuloaga. BRAZIL. Bahia: Mun. Rio de Contas: Pico das Almas, vertente leste, subida do pico do campo do Queiroz, *Harley et al. 26321* (isotype, SI).

P. animarum Renvoize. BRAZIL. Bahia: Mucugé, *Giulietti et al. 1537* (SI); Mun. Rio de Contas, subida a Pico das Almas, 13°35′S, 41°41′W, 1450 m, 15 Feb. 1994, *Zuloaga et al. 4848* (SI).

P. bahiense Renvoize. BRAZIL. Bahia: Mun. Rio de Contas, subida a Pico das Almas, *Zuloaga et al. 4850* (SI).

P. chnoodes Trin. BRAZIL. Minas Gerais: Serra do Cipó, entre Posta Palacio e Morro do Pilar, *Vidal 5996* (SI).

P. cipoense Renvoize & Send. BRAZIL. Minas Gerais: Rodovia de Cardeal Mota a Conceiçao do Mato Dentro, BR-010, Serra do Cipó, Km 118, *Zuloaga & Morrone 4694* (SI); Mpio. Jaboticatubas, ao longo da rodavia Lagoa Santa, Conceição do Mato Dentro, Diamantina, *Sendulsky et al. 389* (paratype, SI).

P. durifolium Renvoize & Zuloaga. BRAZIL. Bahia: Mun. Rio de Contas: 11 km ao N da cidade na estrada para o

povoado de Mato Grosso, 13°30′S, 41°52′W, *Harley et al. 26053* (isotype, SI).

P. euprepes Renvoize. BRAZIL. Bahia: Mun. Palmeiras, Pai Inacio, Morro do Pai Inacio, campo rupestre, *Zuloaga et al. 4783* (SI). Minas Gerais: Mun. Jaboticatubas, Km 115 ao longo da rodovia Lagoa Santa–Conceição do Mato Dentro–Diamantina, *Joly et al. 861* (SI); Rodovia de Cardeal Mota a Conceição do Mato Dentro, BR-010, Serra do Cipó, Km 118, *Zuloaga & Morrone 4696* (SI).

P. lagostachyum Renvoize & Zuloaga. BRAZIL. Bahia: Morro do Chapéu, *Pereira 2139* (SI).

P. loreum Trin. BRAZIL. Minas Gerais: Rodovia de Cardeal Mota a Conceição do Mato Dentro, BR-010, 22–24 km de Cardeal Mota, *Zuloaga & Morrone 4597* (SI); Mpio. de Jaboticatubas, ao longo da rodovia Lagoa Santa–Conceição do Mato Dentro–Diamantina, *Sendulsky et al. 448* (SI).

P. lutzii Swallen. BRAZIL. Rio de Janeiro: Pedra Bonita, *Castellanos s.n.* (SI).

P. molinioides Trin. BRAZIL. Minas Gerais: Rodovia de Cardeal Mota a Conceição do Mato Dentro, BR-010, Serra do Cipó, Km 121, *Zuloaga & Morrone 4701* (SI).

P. poliophyllum Renvoize & Zuloaga. BRAZIL. Minas Gerais: Serra de Ouro Branco, *Zuloaga s.n.* (SI).

P. restingae Renvoize & Zuloaga. BRAZIL. Espirito Santo: Guarapari, Km 32 da ES060–Setiba, *Silva 579* (SI).

P. subtiramulosum Renvoize & Zuloaga. BRAZIL. Distrito Federal: Reserva Ecol. do IBGE, próximo ao Córrego Taquara, *Fonseca & Alvarenga 2109* (SI); APA Gama–Cabeca de veado. Cristo Redentor, *Filgueiras & Zuloaga 2132* (SI).

P. trinii Kunth. BRAZIL. Bahia: Mun. Rio de Contas: Pico das Almas, vertente leste, Campo do Queiroz, *Harley et al. 26662* (CEPEC); Pico das Almas, vertente leste, na parte N do vale abaixo do pico, *Harley et al. 26203* (SI); Distrito de Serra Grande, Itacaré estrada que liga Serra Grande, ramal 13 que lleva ao Campinho Cheiroso, *Amorim et al. 727* (CEPEC). Distrito Federal: Restinga de Jacarepaguá, *Duarte 5014* (SI).

P. raginiviscosum Renvoize & Zuloaga. BRAZIL. Bahia: Mun. Ilheus, ruta de Olivença a Una, 17 km de Olivença, en borde de selva, *Zuloaga et al. 4853* (SI); Mun. Palmeiras, Pai Inacio, Morro do Pai Inacio, campo rupestre, *Zuloaga et al. 4781* (SI); Mun. Rio de Contas, Pico das Almas, vertente leste, Trilho Faz. Silvina-Queiroz, *Harley et al. 25772* (isotype, SI).

A SYNOPSIS OF AN EXPANDED *SOLMS-LAUBACHIA* (BRASSICACEAE), AND THE DESCRIPTION OF FOUR NEW SPECIES FROM WESTERN CHINA[1]

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Abstract

Sequence data from the nuclear ribosomal ITS region and the chloroplast *trnL-F* were used to examine the generic delimitations of *Solms-laubachia* Muschl., *Christolea* Cambess., *Desideria* Pamp., *Leiospora* (C. A. Mey.) F. Dvořák, and *Phaeonychium* O. E. Schulz. *Solms-laubachia*, *Desideria*, and *P. jafrii* Al-Shehbaz formed a well-supported monophyletic clade, with *Christolea* as sister group. However, both *Solms-laubachia* and *Desideria* were polyphyletic, as they appeared in more than two positions in that clade, within which *P. jafrii* was embedded. The results are consistent with those based on sequences of plastid maturase (*mat*K) and the nuclear chalcone synthase (*Chs*). SEM survey further reveals that the seed epidermis micromorphology of *D. baiogoinensis* (K. C. Kuan & C. H. An) Al-Shehbaz is most similar to that of *S. lanata* Botsch. Based on these findings, a comprehensive synopsis of an expanded *Solms laubachia* with 26 species is presented; all species of *Desideria* and *P. jafrii* are transferred to *Solms-laubachia* resulting in 12 new combinations: *S. baiogoinensis* (K. C. Kuan & C. H. An) J. P. Yue, Al-Shehbaz & H. Sun, *S. flabellata* (Regel) J. P. Yue, Al-Shehbaz & H. Sun, *S. haranensis* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, *S. himalayensis* (Cambess.) J. P. Yue, Al-Shehbaz & H. Sun, *S. incana* (Ovcz.) J. P. Yue, Al-Shehbaz & H. Sun, *S. jafrii* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, *S. linearis* (N. Busch) J. P. Yue, Al-Shehbaz & H. Sun, *S. mieheorum* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, *S. mirabilis* (Pamp.) J. P. Yue, Al-Shehbaz & H. Sun, *S. nepalensis* (H. Hara) J. P. Yue, Al-Shehbaz & H. Sun, *S. prolifera* (Maxim.) J. P. Yue, Al Shehbaz & H. Sun, and *S. stewartii* (T. Anderson) J. P. Yue, Al-Shehbaz & H. Sun. Furthermore, four new species of *Solms-laubachia* are described from western China: *S. angustifolia* J. P. Yue, Al-Shehbaz & H. Sun, *S. grandiflora* J. P. Yue, Al-Shehbaz & H. Sun, and *S. sunhangiana* J. P. Yue & Al-Shehbaz (all from Sichuan Province), and *S. calcicola* J. P. Yue, Al-Shehbaz & H. Sun (from Xizang). Both *S. linearifolia* (W. W. Sm.) O. E. Schulz and *S. mirabilis* are lectotypified.

Key words: Brassicaceae, China, *Christolea*, *Desideria*, *Leiospora*, ITS, IUCN Red List, *Phaeonychium*, *Solms-laubachia*, *trnL-F*.

Solms-laubachia Muschl. (Brassicaceae) is a Sino-Himalayan endemic genus characterized by the perennial habit, entire leaves, trichomes absent or simple, latiseptate fruits detached at maturity from the pedicel, entire capitate stigmas, and rounded replum concealed by the strongly angled valve margins (Lan & Cheo, 1981; Al-Shehbaz & Yang, 2001). Except for *S. platycarpa* (Hook. f. & Thomson) Botsch., which also occurs in Bhutan and Sikkim, the other eight species previously described in the genus are highly restricted to the alpine areas of northwestern Yunnan, western Sichuan, and eastern Tibet, where they grow

[1] Portions of this paper are taken from a thesis entitled "Phylogeny of the genus *Solms-laubachia* (Brassicaceae)" and submitted by the first author in partial fulfillment of a doctorate degree to the Department of Phytogeography and Ecology of Kunming Institute of Botany, Chinese Academy of Sciences. The new species were collected during field investigations in the Hengduan Mountain region of China, with support from the U.S. National Science Foundation (grant no. DEB-0321846 to David E. Boufford) and the National Natural Science Foundation of China (grant nos. 30625004, 40332021, 40771073, and 30420120019 to H. Sun). Lab work was partially supported by the Innovation Project of the Chinese Academy of Sciences (KSCX2-YW-Z-030) and a Mercer Fellowship from the Arnold Arboretum to J. P. Yue. Fieldwork in Xinjiang by I. Al-Shehbaz was supported by the National Geographic Society (grant 7405-03). We thank Jason R. Grant for providing DNA of three accessions of *Desideria* and one of *Leiospora*. We are grateful to Victoria C. Hollowell, Beth Parada, and Allison Brock for their editorial advice, to Barbara Neuffer and Karol Marhold for their critical reviews of the manuscript, and to Fred Keusenkothen for lettering the seed figures. Last, but certainly not least, we thank the directors and curators of the herbaria cited.

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on scree slopes and in rock crevices (Lan & Cheo, 1981; Al-Shehbaz & Yang, 2001).

Solms-laubachia was placed by Schulz (1936) in the tribe Matthioleae, a disposition followed by several Chinese taxonomists (e.g., Kuan, 1985; Lan, 1987; An, 1995; Li, 1995; Huang, 1997; Tan et al., 1999). However, Schulz's tribal classification of the Brassicaceae has been considered as highly artificial (Hedge, 1976; Al-Shehbaz, 1984; Koch et al., 2003; Mitchell-Olds et al., 2005; Al-Shehbaz et al., 2006). Janchen (1942) united the Matthioleae with the Hesperideae, a position followed by Al-Shehbaz (1984) who adopted the earlier tribal name Anchonieae and further suggested the exclusion of *Solms-laubachia* and other genera from the combined tribe (Al-Shehbaz, 1988). In their broadly sampled molecular phylogenetic study on the Brassicaceae, Beilstein et al. (2006) showed that *Solms-laubachia* (with *S. zhongdianensis* J. P. Yue, Al-Shehbaz & H. Sun as the representative) falls within a well-supported monophyletic clade containing species of *Braya* Sternb. & Hoppe, *Desideria* Pamp., *Euclidium* W. T. Aiton, *Malcolmia* W. T. Aiton, *Neotorularia* Hedge & J. Léonard, *Sisymbriopsis* Botsch. & Tzvelev, *Rhammatophyllum* O. E. Schulz, *Shangrilaia* Al-Shehbaz, J. P. Yue & H. Sun, and *Tetracme* Bunge. Based on the molecular results and morphological evidence, Al-Shehbaz et al. (2006) tentatively assigned these 10 genera and 15 others within the tribe Euclidieae. However, the generic delimitation within this tribe needs further study since some genera (e.g., *Neotorularia*, *Sisymbriopsis*) have been shown to be polyphyletic (Warwick et al., 2004, 2007).

Yue et al. (2006) used sequences of the nuclear chalcone synthase (*Chs*) and plastid maturase (*matK*) to study the phylogenetic relationships of *Solms-laubachia* within the Brassicaceae. They found that the four *Desideria* species, *D. baiogoinensis* (K. C. Kuan & C. H. An) Al-Shehbaz, *D. himalayensis* (Cambess.) Al-Shehbaz, *D. linearis* (N. Busch) Al-Shehbaz, and *D. stewartii* (T. Anderson) Al-Shehbaz, as well as *Phaeonychium jafrii* Al-Shehbaz were nested within *Solms-laubachia* in a well-supported clade. Therefore, their data support an expanded *Solms-laubachia*, including *Desideria* and *P. jafrii*. The present paper aims to further test the relationships of *Solms-laubachia*, *Desideria*, and *P. jafrii* using sequences of the nuclear ribosomal ITS and the chloroplast non-coding *trnL-F* region. Additionally, seed epidermis of 13 *Solms-laubachia* species was surveyed under SEM. Finally, a comprehensive synopsis of *Solms-laubachia* is presented in this paper based on the molecular and morphological data.

Materials and Methods

Plant Material

Plant material used in this study is listed in Table 1. The ITS sequences were obtained from 19 species (22 accessions), including 13 of *Solms-laubachia* (17 accessions, including four new species), two species of *Desideria*, one species of *Christolea* Cambess., one accession each of *Leiospora pamirica* (Botsch. & Vved.) Botsch. & Pachom. and *Phaeonychium jafrii*. Published ITS sequences of *D. incana* (Ovcz.) Al-Shehbaz and *D. prolifera* (Maxim.) Al-Shehbaz were also included in the analyses (Table 1). The *trnL-F* sequences were obtained from 21 species (24 accessions), including 12 species of *Solms-laubachia* (16 accessions), four species of *Desideria* (five accessions), one accession of *P. jafrii*, one species of *Christolea*, and two species of *Leiospora* (C. A. Mey.) F. Dvořák. *Leiospora* species were chosen as outgroups because this genus was sister to the clade containing species of *Christolea*, *Solms-laubachia*, *Desideria*, and *Phaeonychium* O. E. Schulz (Yue et al., 2006). Taxa whose seeds were observed under SEM are listed in Table 1 and marked with an asterisk.

DNA Extraction, PCR, and Sequencing

Total genomic DNAs were extracted from silica gel–dried leaves using a Qiagen DNeasy Plant Mini Kit (Santa Clarita, California, U.S.A) following the manufacturer's protocol. Genomic DNA from three accessions of *Desideria* and one of *Leiospora* were kindly provided by Jason R. Grant (Université de Neuchâtel, Switzerland).

Nuclear ribosomal DNA ITS. The nuclear ribosomal DNA (nrDNA) ITS region was amplified using primers ITS-4 of White et al. (1990) and ITS-leu of Baum et al. (1998). A 25-μl polymerase chain reaction (PCR) included 2.5 μl of 10× Taq polymerase buffer, 4 μl of dNTP (2.5 mM), 3 μl of $MgCl_2$ (25 mM), 1 μl of each primer (10 μM), 0.3 μl of Taq polymerase (5 units/μl), 2 μl of dimethyl sulfoxide (DMSO), and 20–50 ng DNA. The PCR program included a 3-min. hotstart and 34 cycles followed by an additional 10 min. at 72°C. Each cycle consisted of 45 sec. denaturing at 94°C, 70 sec. annealing at 55°C–57°C, and a 1-min. extension at 72°C. PCR products were gel-purified using a Qiagen Gel Purification Kit, following the manufacturer's protocol. For sequencing, the same primers as for PCR were used; for some taxa, ITS-2 and ITS-3 of White et al. (1990) were used as well.

Table 1. Vouchers and sources of species used in this study. An asterisk (*) indicates the taxa also studied for seed epidermis under SEM. Dagger symbol (†) indicates sequence obtained from the GenBank database.

Taxon and accession	Voucher	Source	ITS	*trnL-F*
**Solms-laubachia angustifolia* J. P. Yue, Al-Shehbaz & H. Sun	*Yue 0250* (KUN)	China, Sichuan, Daocheng	DQ523420	DQ523319
**Solms-laubachia baiogoinensis* (K. C. Kuan & C. H. An) J. P. Yue, Al-Shehbaz & H. Sun [= *Desideria baiogoinensis*]	*Yue 0246* (KUN)	China,Tibet, Mozhugongka	DQ523416	DQ523315
**Solms-laubachia calcicola* J. P. Yue, Al-Shehbaz & H. Sun	*Boufford et al. 31975* (A)	China, Tibet, Riwoqe	DQ523421	DQ523320
**Solms-laubachia eurycarpa* (Maxim.) Botsch., 1	*Yue 0249* (KUN)	China, Sichuan, Yading	DQ523404	DQ523303
Solms-laubachia eurycarpa (Maxim.) Botsch., 2	*Yue 0158* (KUN)	China, Yunnan, Deqing	DQ523405	DQ523304
**Solms-laubachia grandiflora* J. P. Yue, Al-Shehbaz & H. Sun	*Boufford et al. 30727* (A)	China, Sichuan, Xiangcheng	DQ523419	DQ523318
Solms-laubachia himalayensis (Cambess.) J. P. Yue, Al-Shehbaz & H. Sun [= *Desideria himalayensis*]	*McBenth 1486* (E)	Nepal	–	DQ523327
Solms-laubachia incana (Ovez.) J. P. Yue, Al-Shehbaz & H. Sun [*Desideria incana*]	–		†AJ628335	
Solms-laubachia jafrii (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun [= *Phaeonychium jafrii*]	*Yue 0233* (KUN)	China, Tibet, Lhasa	DQ523422	DQ523321
**Solms-laubachia lanata* Botsch., 1	*Yue 0234* (KUN)	China, Tibet, Lhasa	DQ523409	DQ523308
Solms-laubachia lanata Botsch., 2	*Yue 0237* (KUN)	China, Tibet, Lhasa	DQ523410	DQ523309
**Solms-laubachia linearifolia* (W. W. Sm.) O. E. Schulz	*Yue 0157* (KUN)	China, Yunnan, Deqing	DQ523414	DQ523313
Solms-laubachia linearis (N. Busch) J. P. Yue, Al-Shehbaz & H. Sun, 1 [= *Desideria linearis*]	*Bartholomew et al. 9549* (MO)	China, Xinjiang	DQ523417	DQ523316
Solms-laubachia linearis (N. Busch) J. P. Yue, Al-Shehbaz & H. Sun, 2 [= *Desideria linearis*]	*Stainton 3055* (E)	Pakistan		DQ523329
**Solms-laubachia minor* Hand.-Mazz.	*Yue 0379* (KUN)	China, Sichuan, Yanyuan	DQ523418	DQ523317
**Solms-laubachia platycarpa* (Hook. f. & Thomson) Botsch., 1	*Yue 0238* (KUN)	China, Tibet, Dangxion	DQ523408	DQ523307
Solms-laubachia platycarpa (Hook. f. & Thomson) Botsch., 2	*Yue 0239* (KUN)	China, Tibet, Dangxion	DQ523407	DQ523306
Solms-laubachia prolifera (Maxim.) J. P. Yue, Al-Shehbaz & H. Sun [= *Desideria prolifera*]		–	†AJ628333	–
**Solms-laubachia pulcherrima* Muschl.	*Yue 0153* (KUN)	China, Yunnan, Lijian	DQ523411	DQ523310
**Solms-laubachia retropilosa* Botsch., 1	*Yue 0162* (KUN)	China, Sichuan, Xiancheng	DQ523413	DQ523312
Solms-laubachia retropilosa Botsch., 2	*Yue 0246* (KUN)	China, Tibet, Zuogong	DQ523412	DQ523311
Solms-laubachia stewartii (T. Anderson) J. P. Yue, Al-Shehbaz & H. Sun [= *Desideria stewartii*]	*McBeath 2105* (E)	India		DQ523328
Solms-laubachia sunhangiana J. P. Yue & Al-Shehbaz	*Boufford et al. 33461* (A)	China, Sichuan, Jiulong	EU186027	–
**Solms-laubachia xerophyta* (W. W. Sm.) H. F. Comber	*Yue 0251* (KUN)	China, Yunnan, Zhongdian	DQ523406	DQ523305
**Solms-laubachia zhongdianenis* J. P. Yue, Al-Shehbaz & H. Sun	*Yue 0156* (KUN)	China, Yunnan, Zhongdian	DQ523415	DQ523314
Outgroup taxa				
Christolea crassifolia Cambess.	*Bartholomew et al. 9499* (MO)	China, Xinjiang	DQ523423	DQ523322
Leiospora exscapa (C. A. Mey.) F. Dvořák	*Murray et al. 457* (ALA)	Russia	–	DQ523330
Leiospora pamirica (Botsch. & Vved.) Botsch. & Pachom.	*Bartholomew et al. 9790* (MO)	China, Xinjiang	DQ523424	DQ523323

Plastid trnL-F. The primer pair c and f of Taberlet et al. (1991) was used to amplify the *trnL* intron and the *trnL-trnF* intergenic spacer. A 50-µl PCR reaction included 50–100 ng DNA, 5 µl of 10× Taq polymerase buffer, 4 µl of dNTP (2.5 mM), 4 µl of $MgCl_2$ (25 mM), 2 µl of each primer (10 µM), and 0.5 µl of Taq polymerase (5 units/µl). The PCR profile used to amplify the *trnL-F* region included a 3-min. hotstart and 34 cycles (94°C for 1 min., 55°C for 1 min., 72°C for 2 min.), and a final extension at 72°C for 7 min. PCR products were purified using the method described above. Sequencing of the region was done using the PCR primers as well as primers d and e of Taberlet et al. (1991).

Sequences were analyzed using an automated ABI Genetic Analyzer 3100 (GMI, Inc., Ramsey, Minnesota, U.S.A.), then edited using Sequencher (version 3.0, Gene Codes Corp., Inc., Ann Arbor, Michigan, U.S.A.), and then aligned using ClustalX version 1.83 (PC version) (Thompson et al., 1997), followed by manual adjustments.

PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed for the data sets using maximum parsimony (MP) (Farris et al., 1970; Fitch, 1971). The heuristic searches for MP analysis were conducted using PAUP 4.0b10 (Swofford, 2002) with the following conditions: random sequence addition with 1000 replicates and 10 trees held at each step, tree bisection-reconnection (TBR) branch swapping, MULPARS on, and the steepest descent off. Character state changes were unordered and equally weighted in the analysis. Gaps were treated as missing data. Indels in the *trnL-F* alignment were coded following Simmons and Ochoterena (2000). Bootstrap (BS) and jackknife analyses of 1000 replicates were performed respectively to estimate the support for individual clades using heuristic tree search options as in the MP analyses.

It was not possible to obtain the ITS sequences for *Desideria himalayensis*, *D. stewartii*, *D. linearis*, and *Leiospora exscapa* (C. A. Mey.) F. Dvořák, nor produce the *trnL-F* sequences for *D. prolifera*, *D. incana*, and *Solms-laubachia sunhangiana* J. P. Yue & Al-Shehbaz. Except for these seven species, the ITS and *trnL-F* sequences of all other exemplar taxa were combined into a data set, and the incongruence length difference (ILD) test (Farris et al., 1994, as implemented in PAUP) was used to assess potential conflicts between the phylogenetic signals from the two different DNA data sets. For that test, 1000 replicates were analyzed with a heuristic search, each with 10 random sequence addition replicates. To ensure the accuracy of the *P* value in the ILD test, only parsimony informative positions were used.

SEED EPIDERMIS

Seeds of each species were mounted on aluminum stubs using double-sided adhesive and sputter-coated with gold-palladium. Observations were made using a KYKY-10000B (Science Instrument Company, Beijing, China) electronic microscope at 15 kV. Descriptive terminology of seed-coat epidermis follows Murley (1951).

Results and Discussion

ITS SEQUENCE DATA

Twenty-four sequences of the ITS region, including the 5.8S gene, were used in the analysis (Table 1). The multiple alignment of the ITS region produced a data set of 622 bp, of which 514 bp were uninformative, 65 bp were variable but not parsimony informative, and only 43 bp were potentially parsimony informative. The ITS-1 region was variable at 59 of the 277 sites (21.3%), the ITS-2 was variable at 45 of the 221 sites (20.4%), and the 5.8S gene was variable at only 4 of the 124 sites (3.2%).

The sequences were similar in length, and the alignment only required six indels with one, two, or three bp. ITS sequence divergence ranged from 5.7%–9.2% between the ingroup and outgroup and 0%–7.5% within the ingroup. The sequences of two accessions of *Solms-laubachia lanata* Botsch. were identical, and those of the single accessions of *S. calcicola* J. P. Yue, Al-Shehbaz & H. Sun and *S. sunhangiana* were indistinguishable.

The MP analysis yielded three most parsimonious trees (MPTs) of 150 steps (confidence interval [CI] = 0.82, retention index [RI] = 0.77, and rescaled consistency index [RC] = 0.63) (Fig. 1). All sampled species of *Solms-laubachia*, *Desideria*, and *Phaeonychium jafrii* formed a monophyletic clade with 98% BS support. Within this clade, *D. linearis* was basal to the remaining taxa. Eleven species of *Solms-laubachia* formed a core clade with 71% BS support. Within core *Solms-laubachia*, two accessions of *S. retropilosa* Botsch. and one of *S. linearifolia* (W. W. Sm.) O. E. Schulz lay basal to a weakly supported (51%) clade that consisted of three groups, with weak support. *Desideria prolifera* was basal to core *Solms-laubachia*, whereas *D. incana* and *P. jafrii* were basal to these other species, but with weak support.

TRNL-F SEQUENCE DATA

Twenty-five accessions were included in the analysis (Table 1). The multiple alignment of the

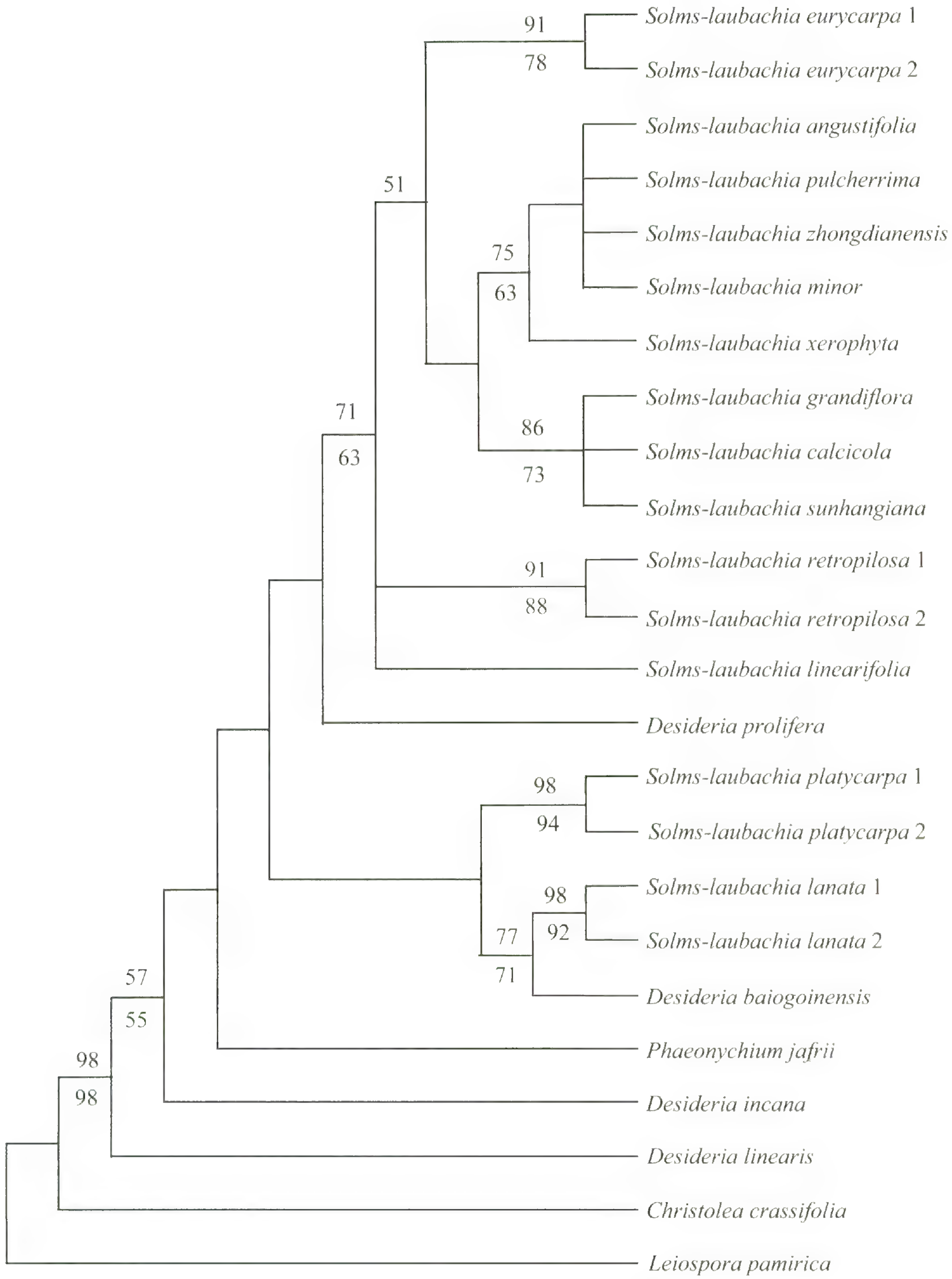

Figure 1. Strict consensus tree of three MPTs based on ITS sequence. Numbers above and below branches are BS and jackknife percentages (values lower than 50% not shown).

trnL-F region required 42 indels. The data set had 1106 characters. However, a segment of 64 bp (bp 302–365) was excluded because of ambiguous sequence alignment. Of the remaining 1042 characters, 885 were uninformative, 75 were variable but not parsimony informative, and 82 were potentially parsimony informative.

The *trnL-F* sequence divergence ranged from 3.4%–5.0% between the ingroup and outgroup and 0%–3.3% within the ingroup. Identical sequences were detected between two conspecific accessions of *Solms-laubachia platycarpa*.

The MP analysis yielded 2565 MPTs of 193 steps (CI = 0.84, RI = 0.81, and RC = 0.68; cf. Fig. 2). Twelve *Solms-laubachia* species, together with the four *Desideria* species and *Phaeonychium jafrii*, formed a monophyletic clade, with *Christolea crassifolia* Cambess. as sister group with 100% BS support. Within this, *Desideria himalayensis* and *D. stewartii* formed a basal clade with 91% BS support. The remaining species formed less resolved polytomy with only 58% bootstrap support. The two accessions of *S. lanata* were grouped with *S. angustifolia* J. P. Yue, Al-Shehbaz & H. Sun at 60% BS support, while *D. baiogoinensis* occupied a solitary position in the *Solms-laubachia* polytomy.

COMBINED SEQUENCE DATA

The ILD test returned a *P* value of 0.014, which is lower than the general threshold of 0.05 (Farris et al., 1995). However, Sullivan (1996) argued that the significance threshold of 0.05 may be too conservative for the ILD test. Cunningham (1997) pointed out that combining data improved or did not reduce phylogenetic accuracy whenever a *P* value from the ILD test was greater than 0.01. Therefore, we performed an analysis on the combined data set.

The combined data set consists of 1664 characters, among which 1419 were uninformative, 146 were variable but not parsimony informative, and 99 were potentially parsimony informative. Phylogenetic analysis of the combined data set yielded two MPTs of 324 steps (CI = 0.81, RI = 0.74, and RC = 0.57), which have a topology nearly identical to the ITS tree, differing only in the position of *Desideria baiogoinensis* (not shown). In the combined tree, *D. baiogoinensis* clustered with *Solms-laubachia platycarpa* rather than immediately with *S. lanata* as in the ITS strict consensus. However, no relationships received convincing support. With the exception of the basal position of *D. linearis*, which received 84% BS support, the relationships among other ingroups were not convincingly resolved in the combined analysis.

Both ITS and *trnL-F* sequence data revealed that *Solms-laubachia*, *Desideria*, and *Phaeonychium jafrii* formed a monophyletic clade (Figs. 1, 2). However, neither *Solms-laubachia* nor *Desideria* is monophyletic, appearing in more than two positions within the core clade. Therefore, sequences of the ITS and *trnL-F* results support an expanded *Solms-laubachia* that includes *Desideria* and *P. jafrii*, as suggested by Yue et al. (2006). However, neither the separate nor combined analysis could resolve their terminal relationship convincingly. The present study shows rather low-level sequence divergences of ITS (from 0%–5.1%; mean 2.4%) and *trnL-F* (0%–2.6%; mean 1.33%) among *Solms-laubachia* and *Desideria*.

Comparisons of the phylogenetic trees generated from the ITS (Fig. 1) and the *trnL-F* (Fig. 2) showed some discrepancies for the position of a few taxa on either tree. For example, in the ITS tree *Desideria linearis* was basal to all other ingroup taxa, whereas in the *trnL-F* tree it was embedded in a weakly supported polytomy. *Solms-laubachia lanata* formed a sister group with *D. baiogoinensis* in the ITS phylogeny (77% BS), but switched its sister group relationship to *S. angustifolia* (60% BS) in the *trnL-F* phylogeny. These discordances between nuclear and chloroplast trees may give some clues for exploring speciation mechanisms within *Solms-laubachia*. The species with such conflicting relationships between the ITS and *trnL-F* trees might be the result of hybrid speciation followed by inheritance of chloroplast DNA from one parent and fixation of ITS sequences from the other. However, to test this hypothesis one needs to compare well-resolved phylogenetic relationships generated from both chloroplast and nuclear data sets and sequence characteristics. Due to the low sequence divergence and less resolved relationship in the present study, we could not draw reliable conclusions in the speciation context. To better understand the phylogenetic relationship and speciation mechanism within *Solms-laubachia*, both additional independent gene phylogenies and the application of more sensitive molecular markers are needed, and both approaches are in progress.

SEED EPIDERMIS

Seed epidermis of 12 *Solms-laubachia* species and *Desideria baiogoinensis* was studied under SEM (Figs. 3–5). For each species, we checked three seeds from different individuals. Seeds are rugose in *S. platycarpa*, *S. lanata*, *S. retropilosa*, and *D. baiogoinensis* (Fig. 3A–H) and reticulate in the other species (Figs. 4, 5). Within these two seed types, there is a substantial variation in the sculpturing of the anticlinal cell walls and the fine ornamentation on

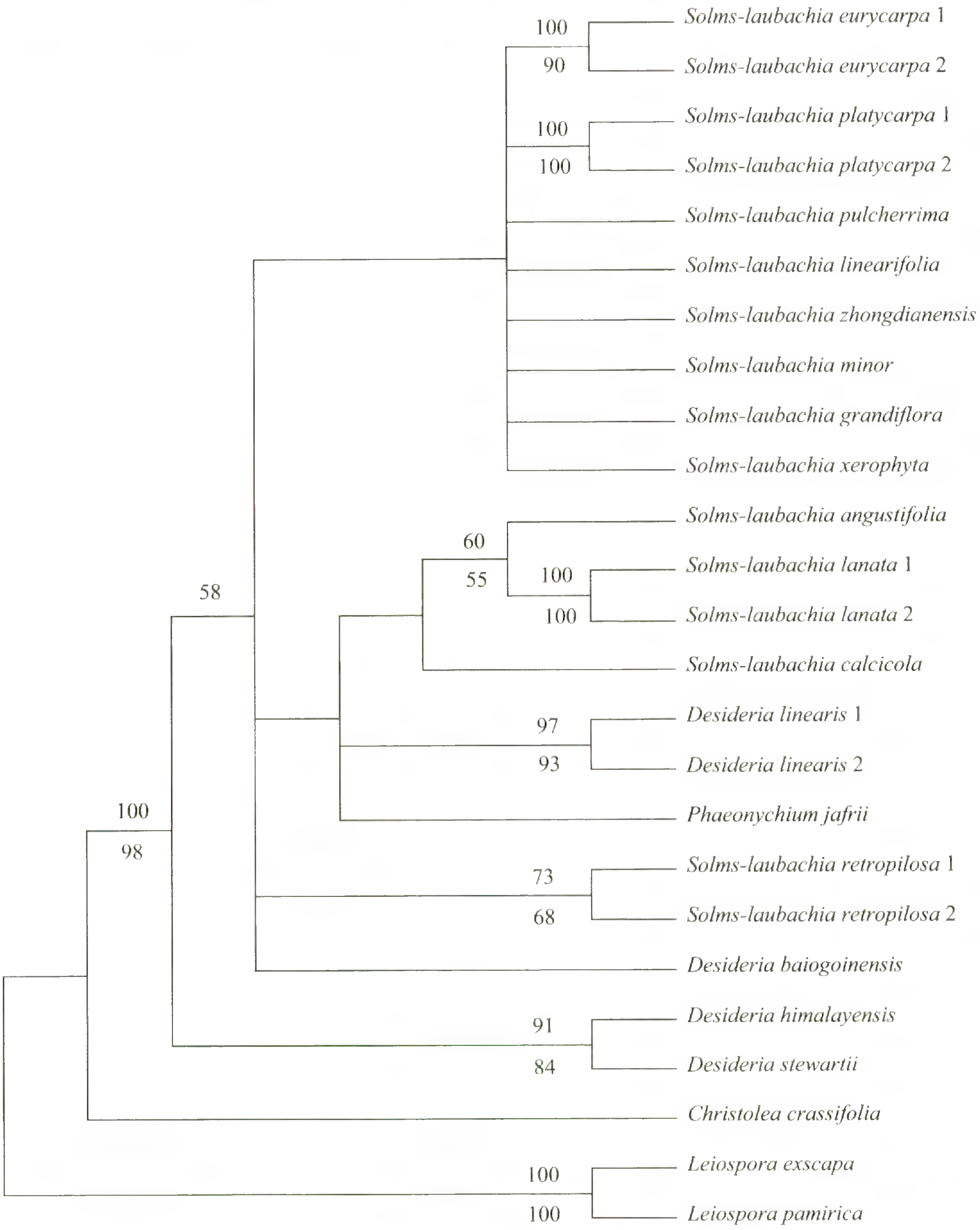

Figure 2. Strict consensus tree of 2565 MPTs based on *trnL-F* sequence. Numbers above and below branches are BS and jackknife percentages (values lower than 50% not shown).

the periclinal cell walls. The seed micro-sculpturing is useful in distinguishing different species with similar gross morphology. For example, *S. grandiflora* J. P. Yue, Al-Shehbaz & H. Sun and *S. linearifolia* are morphologically similar; however, the seed surface of *S. grandiflora* has the compound walls and smooth cells (Fig. 5C, D), distinguished from that of *S. linearifolia* with simple walls and minutely papillate cell surface (Fig. 5A, B). The seed epidermal micromorphology of *D. baiogoinensis* (Fig. 3G, H) was similar to that of *S. lanata* (Fig. 3C, D), with both showing striped periclinal cell walls. This common feature supports the merger of *Desideria* in *Solms-laubachia*. Such a conclusion is consistent with the

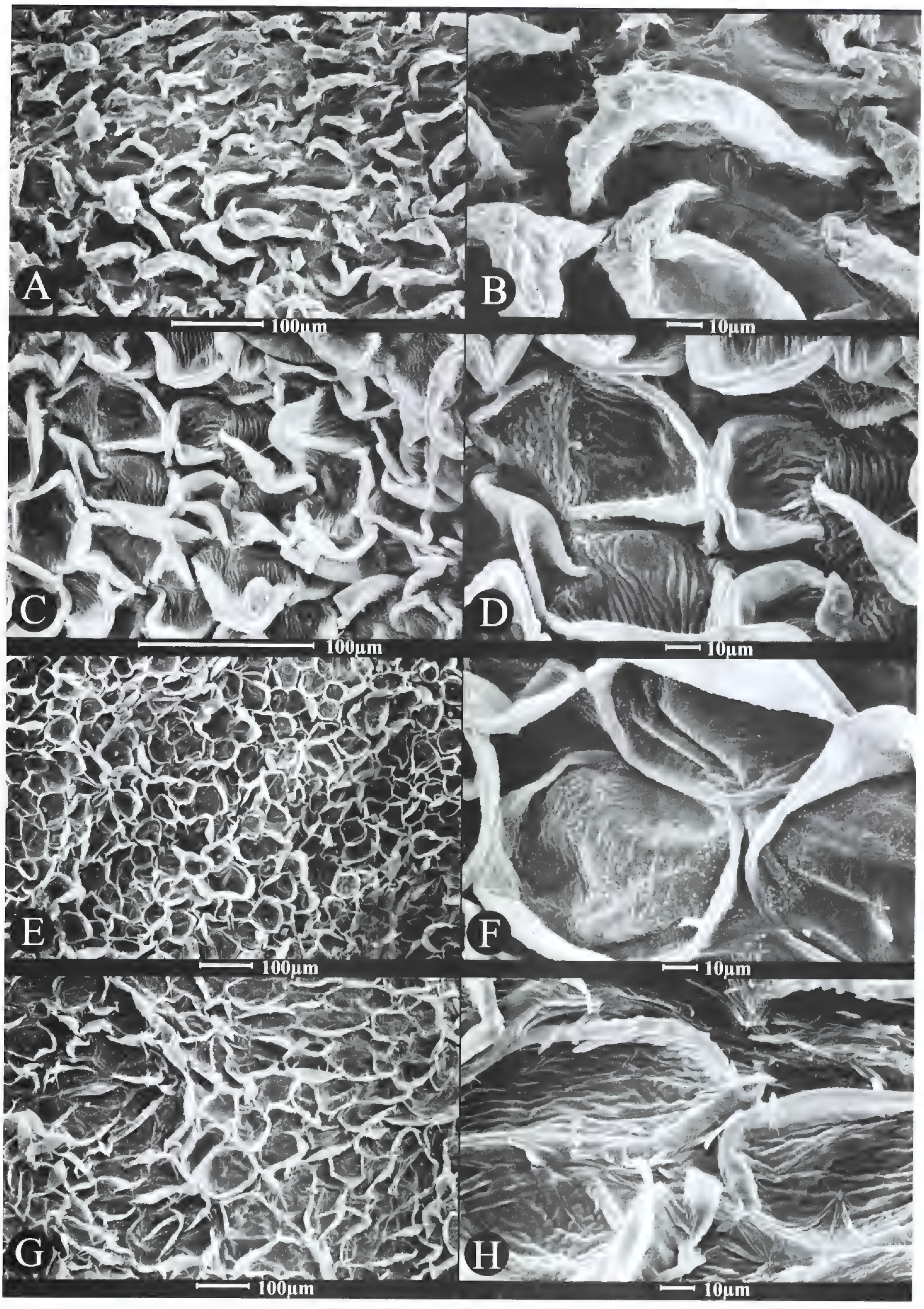

Figure 3. Seed epidermis of *Solms-laubachia* and *Desideria* under SEM. —A, B. *S. platycarpa* (×250; ×1000). —C, D. *S. lanata* (×250; ×1000). —E, F. *S. retropilosa* (×150; ×1000). —G, H. *D. baiogoinensis* (×150; ×1000). Voucher sources for the taxa are represented by asterisks in Table 1.

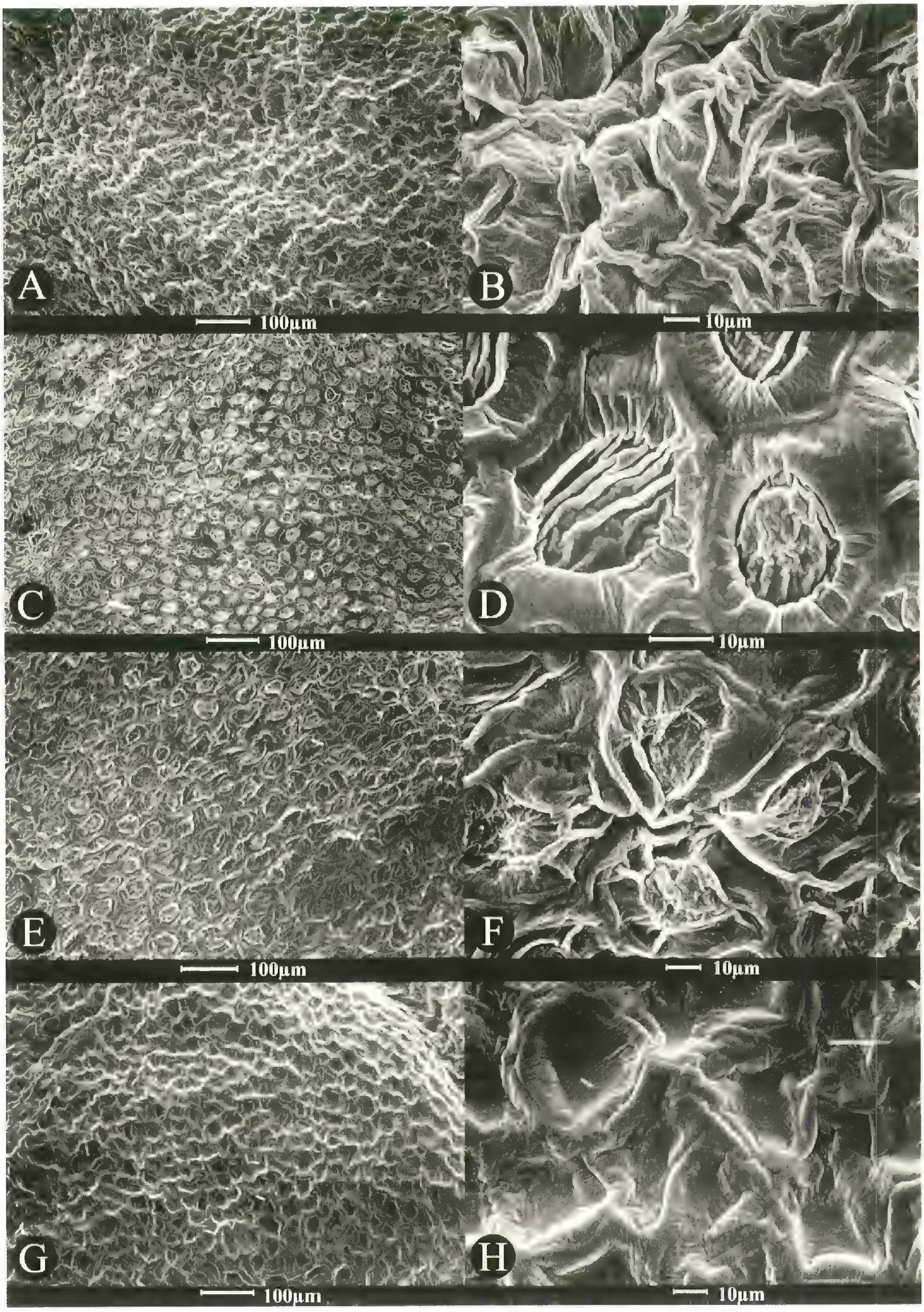

Figure 4. Seed epidermis of *Solms-laubachia* under SEM. A, B. *S. xerophyta* (×150; ×1000). —C, D. *S. angustifolia* (×150; ×1000). —E, F. *S. pulcherrima* (×150; ×1000). —G, H. *S. minor* (×150; ×1000). Voucher sources for the taxa are represented by asterisks in Table 1.

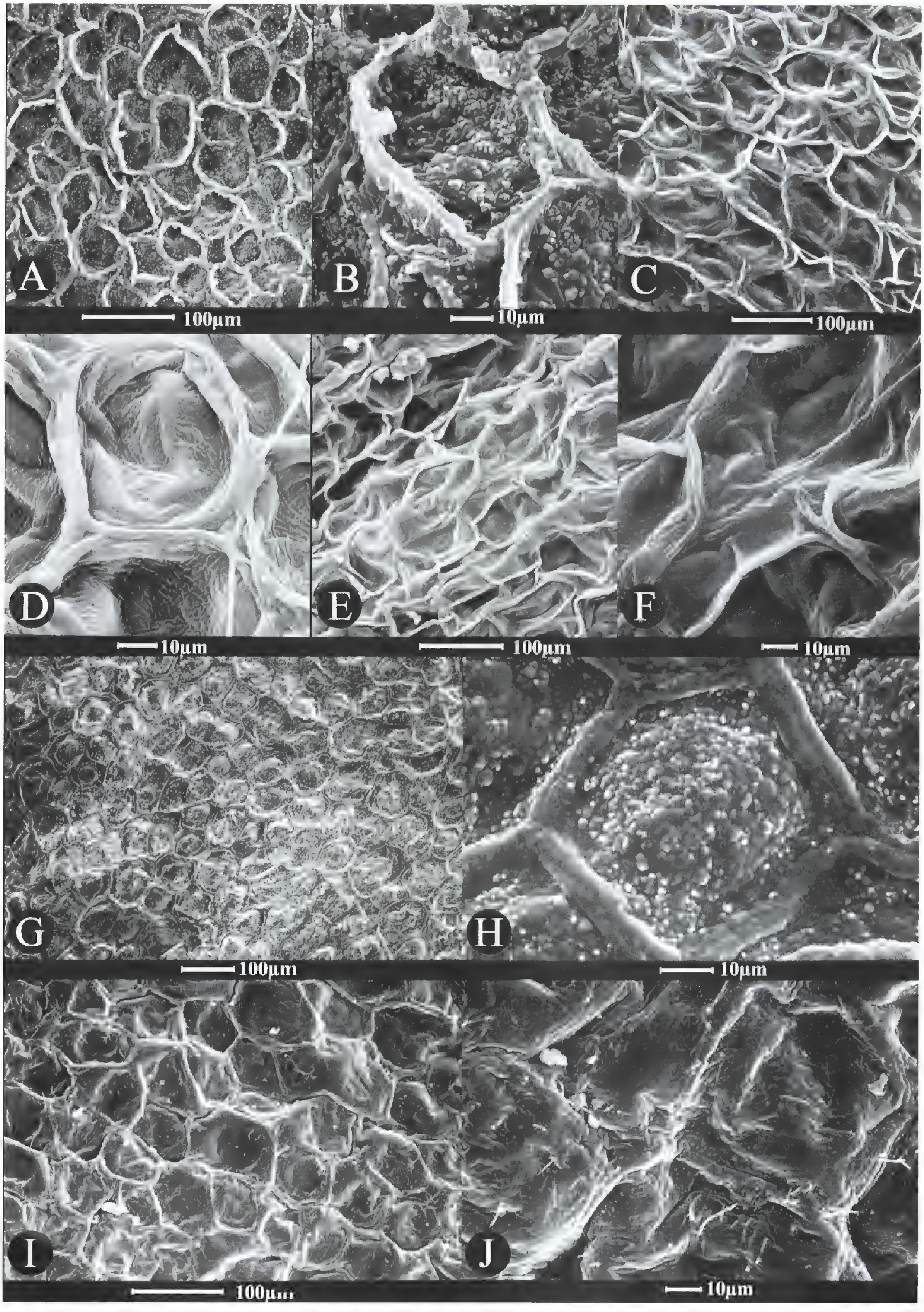

Figure 5. Seed epidermis of *Solms-laubachia* under SEM. —A, B. *S. linearifolia* (×250; ×1200). —C, D. *S. grandiflora* (×250; ×1000). —E, F. *S. calcicola* (×250; ×1000). —G, H. *S. eurycarpa* (×150; ×1500). —I, J. *S. zhongdianensis* (×250; ×1000). Voucher sources for the taxa are represented by asterisks in Table 1.

ITS and *trnL-F* data and previous findings of Yue et al. (2006).

Taxonomic Considerations

Solms-laubachia has been considered by Al-Shehbaz (2001) and Al-Shehbaz and Yang (2001) to be most closely related to *Desideria* (12 species; Al-Shehbaz, 2005) and *Leiospora* (six species; Botschantzev, 1955). All three genera are unique in the Brassicaceae by having fruits tardily dehiscent apically, rectangular in cross section, and readily detached at maturity from the pedicel. Furthermore, the fruit valves are adnate apically to the replum, are strongly angled at the margins, completely conceal the replum, and have prominent marginal veins. Al-Shehbaz et al. (2006) placed these three genera in the tribe Euclidieae, a disposition supported by the recent molecular findings of Warwick et al. (2007).

According to Al-Shehbaz (2001), *Solms-laubachia* differs from *Desideria* by having pinnately (vs. palmately) veined, entire (vs. apically 3- to 9 [to 11]-toothed) leaves and linear-oblong (vs. ovate to oblong) anthers. It differs from *Leiospora* by having wingless (vs. winged) seeds, equal (vs. unequal) sepals with the lateral pair non-saccate (vs. saccate), and entire or 2-lobed stigmas, but the lobes are neither decurrent nor connivent (vs. decurrent and connivent). However, molecular data (Yue et al., 2006; this study) strongly indicate that *Desideria* is polyphyletic and nested within *Solms-laubachia*, and *Solms-laubachia* and *Leiospora* are independent but closely related genera. Warwick et al. (2007) also found *Desideria* to be polyphyletic in relation to *Solms-laubachia*, but this study included only two species each of these two genera. Thus, the above-mentioned differences in leaf morphology between *Solms-laubachia* and *Desideria* are not consistent with the evolutionary relationships reflected by the molecular data. The four molecular markers used in this study and in Yue et al. (2006) show that *Phaeonychium jafrii* is embedded in the *Solms-laubachia–Desideria* clade to form one monophyletic group. Therefore, we transfer below *P. jafrii* and all *Desideria* sensu Al-Shehbaz (2001, 2005) to *Solms-laubachia* and recognize them as *S. jafrii* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, *S. baiogoinensis* (K. C. Kuan & C. H. An) J. P. Yue, Al-Shehbaz & H. Sun, *S. flabellata* (Regel) J. P. Yue, Al-Shehbaz & H. Sun, *S. haranensis* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, *S. himalayensis* (Cambess.) J. P. Yue, Al-Shehbaz & H. Sun, *S. incana* (Ovcz.) J. P. Yue, Al-Shehbaz & H. Sun, *S. linearis* (N. Busch) J. P. Yue, Al-Shehbaz & H. Sun, *S. mieheorum* (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, *S. mirabilis* (Pamp.) J. P. Yue, Al-Shehbaz & H. Sun, *S. nepalensis* (H. Hara) J. P. Yue, Al-Shehbaz & H. Sun, *S. prolifera* (Maxim.) J. P. Yue, Al-Shehbaz & H. Sun, and *S. stewartii* (T. Anderson) J. P. Yue, Al-Shehbaz & H. Sun. An expanded generic description of *Solms-laubachia* and a synopsis and key to all species are given below.

Solms-laubachia Muschl. in Diels, Notes Roy. Bot. Gard. Edinburgh 5: 205. 1912. Type: *Solms-laubachia pulcherrima* Muschl.

Desideria Pamp., Boll. Soc. Bot. Ital. 1926: 111. 1926. TYPE: *Desideria mirabilis* Pamp.

Ermaniopsis H. Hara, J. Jap. Bot. 49: 198. 1974. TYPE: *Ermaniopsis pumila* H. Hara.

Oreoblastus Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 648. 1972. TYPE: *Oreoblastus flabellatus* (Regel) Suslova.

Herbs perennial, sometimes pulvinate, with branched caudex covered with petioles of previous years; trichomes absent or simple, rarely short-stalked, 2-rayed; stems absent or present and simple, leafy or leafless. Basal leaves petiolate, rosulate, simple, entire or 3- to 9(to 11)-toothed, pinnately or palmately veined; cauline leaves absent or similar to basal ones and short petiolate to subsessile, not auriculate. Flowers solitary on long pedicels originating from center of rosette, or in (3 to)6- to 30-flowered, bracteate or ebracteate corymbose racemes elongating or not in fruit. Sepals oblong to ovate, free or united, persistent or deciduous, erect, equal, base of inner pair not saccate, margin membranous or not; petals purple, blue, pink, or rarely white, suborbicular, obovate, to spatulate, apex obtuse to emarginate; claw subequaling or longer than sepals; stamens 6; filaments free, dilated or not at base; anthers oblong-linear to ovate, not apiculate at apex; nectar glands 2 and lateral, or confluent and subtending bases of all filaments; median nectaries absent or present; ovules 6 to 30(to 70) per ovary. Fruit dehiscent silique or silicle, linear, oblong, ovate, or lanceolate, latiseptate, sessile, readily detached at maturity from pedicel, rectangular in cross section; valves papery, reticulate veined, with a prominent midvein and marginal veins, glabrous or pubescent, smooth, adnate with replum at fruit apex, margin angled; replum rounded, concealed by connate valve margins; septum complete or rarely perforated or reduced to a rim, membranous, translucent, rarely absent; style obsolete, rarely to 1 mm long; stigma capitate, entire or 2-lobed, lobes not decurrent. Seeds uniseriate or biseriate, wingless, broadly ovate to suborbicular or oblong, flattened; seed coat reticulate, rugose, or papillate, not mucilaginous when wetted; cotyledons accumbent.

Distribution and habitat. The 26 species of *Solms-laubachia* are distributed in Afghanistan, Bhutan, China (Gansu, Qinghai, Sichuan, Xinjiang, Xizang [Tibet], Yunnan), India (Himachal Pradesh, Kashmir, Punjab, Sikkim), Kyrgyzstan, Nepal, Pakistan, and Tajikistan. They are found at altitudes of (3200–)4000–6200 m and grow primarily on scree slopes and in rock crevices in the alpine areas of the Himalayan, Karakorum, Pamir, and Hengduan mountain ranges.

KEY TO THE SPECIES OF *SOLMS-LAUBACHIA*

1a. Flowers in racemes; stems mostly leafy.
  2a. Leaves entire.
    3a. Stems 1- or 2-leaved; leaf trichomes simple only; petals blue to purple, 10–17 mm long; fruit elliptic to broadly ovate, 8–17 mm wide . . . . . 18. *S. platycarpa*
    3b. Stems leafless; leaf trichomes a mixture of simple and branched; petals white to pink, 6.5–10 mm long; fruit linear, 1.5–2 mm wide . . . . . 10. *S. jafrii*
  2b. Leaves dentate.
    4a. Sepals united, persistent until or after fruit dehiscence; septum reduced to a rim.
      5a. Petals 11–13 × 5–6 mm; raceme 2- to 4-flowered, appearing solitary; Nepal . . . . . 17. *S. nepalensis*
      5b. Petals 5–8 × 1.5–3 mm; raceme with 5 or more flowers; China, Kashmir, Tajikistan . . . 16. *S. mirabilis*
    4b. Sepals free, caducous or rarely persisting until about fruit maturity; septum complete or rarely perforated apically.
      6a. Racemes bracteate throughout.
        7a. Stem and pedicel trichomes forked . . . . . 23. *S. stewartii*
        7b. Stem and pedicel trichomes exclusively simple or absent.
          8a. Fruit lanceolate to linear-lanceolate, (3–)4–6 mm wide; petals (6–)6.5–8 × 3–4 mm; seeds biseriate, (1.5–)1.8–2(–2.3) × 1–1.4 mm . . . . . 8. *S. himalayensis*
          8b. Fruit linear, (0.8–)1–1.7(–2) mm wide; petals 4–5(–5.5) × 1.5–2.5 mm; seeds uniseriate, 0.8–1.1 × 0.5–0.8 mm . . . . . 13. *S. linearis*
      6b. Racemes ebracteate.
        9a. Filaments flattened, subapically toothed; petals 6.5–8 mm long . . . . . 7. *S. haranensis*
        9b. Filaments terete, toothless; petals 10–18 mm long.
          10a. Plants canescent; leaf trichomes almost exclusively branched . . . . . 9. *S. incana*
          10b. Plants not canescent; leaf trichomes exclusively simple.
            11a. Style 5–7 mm long; stigma entire; petals blue; ovules 10 to 16 per ovary . . . 14. *S. mieheorum*
            11b. Style obsolete; stigma prominently 2-lobed; petals purple; ovules 14 to 24 per ovary . . . . . 5. *S. flabellata*
1b. Flowers solitary from a basal rosette; scapes leafless.
  12a. Leaves dentate.
    13a. Fruit ovate to broadly lanceolate, 6–9 mm wide, prominently reticulate veined . . . . . 2. *S. baiogoinensis*
    13b. Fruit linear to linear-lanceolate, 2–5 mm wide, obscurely veined.
      14a. Leaf trichomes forked and simple; replum retrorsely ciliate; valves glabrous; sepals 3–4 mm long; petals 6–7 mm long . . . . . 21. *S. pumila* (Kurz) F. Dvořák
      14b. Leaf trichomes exclusively simple; replum spreading ciliate; valves pubescent; sepals 6–7 mm long; petals 11–14 mm long . . . . . 19. *S. prolifera*
  12b. Leaves entire.
    15a. Leaves gray, densely lanate; seeds rugose.
      16a. Leaf blade lanceolate- to oblanceolate-linear, rarely oblanceolate, retrorsely lanate, 1.5–5(–7) mm wide; fruit valves obscurely veined . . . . . 22. *S. retropilosa*
      16b. Leaf blade broadly spatulate, oblong, to obovate, spreading or antrorsely lanate, (5–)7–17 mm wide; fruit valves prominently veined . . . . . 11. *S. lanata*
    15b. Leaves green, glabrous or pilose; seeds reticulate.
      17a. Sepals 2–3 mm long; fruits 2.8–3.2 mm wide . . . . . 24. *S. sunhangiana*
      17b. Sepals (4.5–)5–8(–11) mm long; fruits (4–)5–18 mm wide.
        18a. Petioles of previous seasons papery; longest leaf blades rarely to 1 cm long . . . . . 26. *S. zhongdianensis*
        18b. Petioles of previous seasons remaining thickened; longest leaf blades (1.5–)2–4.5(–5.5) cm long.
          19a. Leaf blade 0.3–1(–1.5) mm wide, grooved adaxially.
            20a. Petioles not ciliate; leaf blades (2–)2.5–5.5(–7.7) cm long; fruits glabrous, narrowly oblong to linear . . . . . 1. *S. angustifolia*
            20b. Petioles ciliate; leaf blades 0.7–2(–2.5) cm long; fruits pubescent, lanceolate to linear-lanceolate . . . . . 25. *S. xerophyta* (W. W. Sm.) H. F. Comber
          19b. Leaf blade (1.5–)2–16(–23) mm wide, flattened adaxially.
            21a. Leaf blade (7–)10–16(–23) mm wide; petiole strongly thickened, ± corky; sepals united . . . . . 4. *S. eurycarpa* (Maxim.) Botsch.
            21b. Leaf blade (1.5–)2–5(–7) mm wide; petiole slightly thickened; sepals free (sometimes united in *S. linearifolia*).
              22a. Petioles of previous seasons papery . . . . . 12. *S. linearifolia*

22b. Petioles of previous seasons thickened.
23a. Petals pink.
24a. Leaf blade to 1 cm long, glabrous, not ciliate . . . . . 15. *S. minor* Hand.-Mazz.
24b. Leaf blades (1.2–)1.8–4.5(–5.4) cm long, glabrous or sparsely pubescent, ciliate . . . . . 6. *S. grandiflora*
23b. Petals turquoise blue.
25a. Seeds orbicular to broadly ovate; leaf trichomes crisped; caudex loosely branched; plants of scree slopes . . . . . 20. *S. pulcherrima*
25b. Seeds oblong; leaf trichomes if present then straight; caudex compactly branched; plants of rock crevices . . . . . 3. *S. calcicola*

1. Solms-laubachia angustifolia J. P. Yue, Al-Shehbaz & H. Sun, sp. nov. TYPE: China. Sichuan: Yading Xian, Xianuoduoji, 28°23′N, 100°23′E, 4530 m, 10 Sep. 2002, *J. P. Yue 0250* (holotype, KUN!; isotype, MO!).

Herba perennis pulvinata, 2–6 cm alta. Folia basalia rosulata, linearia, (2–)2.5–5.5(–7.7) cm × 0.3–1(–1.3) mm, sparse pubescentia, integra; petiolis 0.4–1.5 cm longis, incrassatis; folia caulina nulla. Pedicelli fructiferi solitarii, 1–1.7 cm longi. Flores ignotes. Fructus lineares vel anguste oblongi, 1.6–4.2(–4.8) cm × (4–)4.5–8 mm, glabri. Semina late ovata vel suborbiculata, 2.1–3.4 × 1.2–2.8 mm.

Herbs perennial, pulvinate, 2–6 cm tall; caudex covered with petioles of previous years. Basal leaves rosulate; blade linear, (2–)2.5–5.5(–7.7) cm × 0.3–1(–1.3) mm, not ciliate, grooved adaxially, sparsely pubescent, base attenuate, margin entire; petioles 0.4–1.5 cm, thickened, not ciliate; cauline leaves absent. Fruiting pedicel solitary from basal rosette, 1–1.7 cm. Flowers not seen; ovules 14 to 22 per ovary. Fruit linear to narrowly oblong, 1.6–4.2(–4.8) cm × (4–)4.5–8 mm; valves glabrous, obscurely reticulate veined; septum complete; style (1–)1.2–1.6 mm; stigma entire to slightly 2-lobed. Seeds biseriate, broadly ovate to suborbicular, 2.1–3.4 × 1.2–2.8 mm. Chromosome number: $2n = 14$ (as *Solms-laubachia xerophyta* in Yue et al., 2004).

Distribution. Narrowly endemic to Sichuan Province, China.

IUCN Red List category. Critically Endangered (CR) (IUCN, 2001).

Discussion. Solms-laubachia angustifolia is closest morphologically to *S. xerophyta* and both have narrow leaves grooved adaxially and with thickened petioles. However, it differs by having non-ciliate petioles, leaf blades (2–)2.5–5.5(–7.7) cm long, and glabrous, narrowly oblong to linear fruits. By contrast, *S. xerophyta* has ciliate petioles, leaf blades 0.7–2(–2.5) cm long, and pubescent, lanceolate to linear-lanceolate fruits. Our previous study (Yue et al., 2006) revealed that *S. angustifolia* is sister to *S. minor*, whereas *S. xerophyta* falls in a separate branch of a polytomy that included the *S. minor*–*S. angustifolia* clade and the *S. pulcherrima*–*S. zhongdianensis* clade. Cytological studies (Yue et al., 2004) on *S. angustifolia* (as *S. xerophyta* therein) and *S. xerophyta* also found distinct differences in their chromosomal karyotypes. Furthermore, all previous records of *S. xerophyta* from Sichuan (e.g., Lan & Cheo, 1981; Tan et al., 1999; Al-Shehbaz & Yang, 2001) represent *S. angustifolia*, and therefore the two taxa occupy different provinces.

Paratypes. CHINA. **Sichuan:** Daocheng Xian, *J. F. C. Rock 16419* (GH), *J. F. C. Rock 16857* (MO, PE), *T. T. Yü 13043* (KUN, PE), *Qinghai-Xizang Team 5818* (PE).

2. Solms-laubachia baiogoinensis (K. C. Kuan & C. H. An) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Christolea baiogoinensis* K. C. Kuan & C. H. An in C. Y. Wu, Fl. Xizangica 2: 388. 1985. *Desideria baiogoinensis* (K. C. Kuan & C. H. An) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 561. 2000. TYPE: China. Xizang: Baiogoin, 5100 m, 18 June 1976, *K. Y. Lang 9460* (holotype, PE!; isotype, PE!).

Distribution. China (Qinghai, Xizang) (see Al-Shehbaz, 2000).

3. Solms-laubachia calcicola J. P. Yue, Al-Shehbaz & H. Sun, sp. nov. TYPE: China. Xizang: Riwoqe Xian, Machala, 31°24′N, 96°40′E, 4770 m, 10 Aug. 2004, *D. E. Boufford, J. H. Chen, S. L. Kelley, J. Li, R. H. Ree, H. Sun, J. P. Yue & Y. H. Zhang 31975* (holotype, KUN!; isotypes, A!, MO!).

Herba perennis pulvinata, 2–5 cm alta. Folia basalia rosulata, linearia vel lanceolata, (1.8–)2.3–4.1(–4.4) cm × 1.9–2.5 mm, glabra vel sparse pubescentia, integra; petiolis (5.7–)6.5–8.9(–9.2) mm longis, incrassatis; folia caulina nulla. Pedicelli fructiferi solitarii, 3–3.5(–5) cm longi. Sepala libera, oblonga, 6.2–6.9 × 2.6–3.2 mm; petala turcosa, obovata, 1.5–1.8 × 0.6–1 cm; ovula 18 ad 28. Fructus anguste ovati vel lanceolati, 1.1–2.3(–2.5) cm × 5.1–8.9 (–9.2) mm, glabri vel sparse pubescentes. Semina oblonga, 2–2.5 × (1.2–)1.3–1.5(–1.7) mm.

Herbs perennial, pulvinate, 2–5 cm tall; caudex compactly branched, covered with petioles of previous

years. Basal leaves rosulate; blade linear to lanceolate, (1.8–)2.3–4.1(–4.4) cm × 1.9–2.5 mm, glabrous or sparsely pubescent with straight trichomes, not ciliate, base attenuate, margin entire; petioles (5.7–)6.5–8.9(–9.2) mm, thickened; cauline leaves absent. Fruiting pedicels solitary from basal rosette, 3–3.5(–5) cm. Sepals free, oblong, 6.2–6.9 × 2.6–3.2 mm; petals turquoise blue, obovate, 1.5–1.8 × 0.6–1 cm; median filaments 3.8–4.5 mm; anthers 1–1.8 mm; ovules 18 to 28 per ovary. Fruit narrowly ovate to lanceolate, 1.1–2.3(–2.5) cm × 5.1–8.9(–9.2) mm, glabrous or sparsely pubescent; valves prominently reticulate veined; septum complete; style 0.5–1.5 mm; stigma slightly 2-lobed. Seeds oblong, uniseriate to biseriate, 2–2.5 × (1.2–)1.3–1.5(–1.7) mm.

Distribution. Narrowly endemic to Xizang Province, China.

IUCN Red List category. Critically Endangered (CR) (IUCN, 2001).

Discussion. *Solms-laubachia calcicola* resembles *S. pulcherrima* in being the only species of the genus with turquoise petals and in having thick petioles and similar flower size, but it differs by having oblong versus orbicular to broadly ovate seeds with coarser seed sculpture (Fig. 5E, F), as well as a thick and compactly branched (vs. slender and loosely branched) caudex. It grows in rock crevices instead of scree slopes, to which *S. pulcherrima* is restricted. Furthermore, molecular data (both ITS and *trnL-F*) clearly show that *S. calcicola* and *S. pulcherrima* fall into two separate clades in both analyses (Figs. 1, 2). The occurrence of turquoise petals in these two somewhat remotely related species would appear that this flower color evolved independently in them.

Paratype. CHINA. **Xizang:** Riwoqe Xian, Machala, 31°24′N, 96°40′E, 4770 m, *T. Y. Tu & J. W. Zhang 599* (KUN).

4. Solms-laubachia eurycarpa (Maxim.) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 169. 1955. Basionym: *Parrya eurycarpa* Maxim., Fl. Tangut. 1: 56. 1889. TYPE: China. Tibet: near Jagem-Gol, 20 July 1884, *N. M. Przewalski s.n.* (holotype, LE!; isotype, PE!).

Solms-laubachia pulcherrima Muschl. var. *latifolia* O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 11: 229. 1931. Basionym: *Solms-laubachia latifolia* (O. E. Schulz) Y. Z. Lan & T. Y. Cheo, Acta Phytotax. Sin. 19: 477. 1981. TYPE: China. SW Sichuan: Mt. Konka, Risonquemba, Konkaling, 4475 m, June–Aug. 1928, *J. F. C. Rock 16870* (lectotype, designated by Al-Shehbaz & Yang, 2001: 375, B!; isotypes, E!, US!, W!).

Solms-laubachia dolicocarpa Y. Z. Lan & T. Y. Cheo, Acta Phytotax. Sin. 19: 477. 1981. TYPE: China. Sichuan: Zoige, 4300 m, 4 Aug. 1960, *Sichuan Drug Plant Expedition 20279* (holotype, NAS [as HJ]!).

Solms-laubachia eurycarpa var. *brevistipes* Y. Z. Lan & T. Y. Cheo, Acta Phytotax. Sin. 19: 476. 1981. TYPE: China. Qinghai: Nanggen, 4400 m, 9 July 1965, *Y. C. Yang 1121* (holotype, HNWP [as HQ]!; isotype, HNWP!).

Solms-laubachia eurycarpa var. *lasiophylla* R. F. Huang in S. W. Liu, Fl. Qinghaiica 1: 510. 1997. TYPE: China. Qinghai: Hen Xian, Waisxan Amishan, 19 July 1972, 4100–4200 m, *P. C. Kuo 9908* (holotype, HNWP [as NWBI]!).

Solms-laubachia gamosepala Al-Shehbaz & G. Yang, Harvard Pap. Bot. 5: 380. 2001. TYPE: China. Yunnan: Deqen, Beima Shan, N side of rd., limestone scree, 28°23′N, 99°01′E, 4700 m, 30 June 1994, *Alpine Garden Society Expedition to China ACE 855* (holotype, K!).

Distribution. China (Gansu, Qinghai, Sichuan, Xizang, Yunnan) (see Al-Shehbaz & Yang, 2001).

5. Solms-laubachia flabellata (Regel) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Parrya flabellata* Regel, Bull. Soc. Imp. Naturalistes Moscou 43: 261. 1870. *Ermania flabellata* (Regel) O. E. Schulz, Bot. Jahrb. Syst. 66: 98. 1933. *Christolea flabellata* (Regel) N. Busch in Komarov, Fl. URSS 8: 330. 1939. *Oreoblastus flabellatus* (Regel) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 651. 1972. *Desideria flabellata* (Regel) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 558. 2000. TYPE: [Kyrgyzstan]. southern Tian Shan, Dschaman-Daban, s.d., *Sewerzow s.n.* (holotype, LE!).

Christolea pinnatifida R. F. Huang, Acta Phytotax. Sin. 35: 556. 1997. TYPE: China. Qinghai: Maqen, Anyemaqen Mtn., 4800 m, 25 June 1981, *R. F. Huang CG-81-154* (holotype, HNWP!).

Distribution. Afghanistan, China (Qinghai, Xinjiang), Kyrgyzstan, Tajikistan (see Al-Shehbaz, 2000).

6. Solms-laubachia grandiflora J. P. Yue, Al-Shehbaz & H. Sun, sp. nov. TYPE: China. Sichuan: Xiancheng Xian, Rizhaosheng Shan, 29°6′N, 99°41′32″E, 4650 m, 15 July 2004, *D. E. Boufford, J. H. Chen, S. L. Kelley, J. Li, R. H. Ree, H. Sun, J. P. Yue & Y. H. Zhang 30727* (holotype, KUN!; isotypes, A!, MO!).

Herba perennis pulvinata, 3–7 cm alta. Folia basalia rosulata, linearia vel lineari-lanceolata, (1.2–)1.8–4.5(–5.4) cm × 1.5–2.3(–2.9) mm, glabra vel sparse pubescentia, integra; petiolis (5.2–)6.8–8.2(–10.2) mm longis, incrassatis; folia caulina nulla. Pedicelli fructiferi solitarii, 1.6–2.5(–3.2) cm longi. Sepala libera vel connata, oblonga, 6.1–7.7 × 2–2.9 mm; petala purpurea, obovata, 1.6–2.5 × (0.6–)0.8–0.9(–1.2) cm; ovula 14 ad 24. Fructus oblongi vel oblongo-lineares, 1–3.4(–4.8) cm × 4–11 mm, glabri vel sparse pubescentes. Semina late ovata vel suborbiculata, 2.4–3.6 × (1.6–)1.8–2.8(–3) mm.

Herbs perennial, pulvinate, 3–7 cm tall; caudex covered with petioles of previous years. Basal leaves

rosulate; blade linear to linear-lanceolate, (1.2–)1.8–4.5(–5.4) cm × 1.5–2.3(–2.9) mm, glabrous or sparsely pubescent, ciliate, base attenuate, margin entire; petioles (5.2–)6.8–8.2(–10.2) mm, thickened; cauline leaves absent. Fruiting pedicels solitary from basal rosette, 1.6–2.5(–3.2) cm. Sepals free or united, oblong, 6.1–7.7 × 2–2.9 mm; petals purple, obovate, 1.6–2.5 × (0.6–)0.8–0.9(–1.2) cm; median filaments 4.5–5.6 mm; anthers 1.3–2 mm; ovules 14 to 24 per ovary. Fruit oblong to oblong-linear, 1–3.4(–4.8) cm × 4–11 mm, glabrous or sparsely pubescent; valves obscurely reticulate veined; septum complete; style 1.1–2.1 mm; stigma slightly 2-lobed. Seeds biseriate, broadly ovate to suborbicular, 2.4–3.6 × (1.6–)1.8–2.8(–3) mm.

Distribution. Narrowly endemic to Xiancheng Xian in Sichuan Province, China.

IUCN Red List category. Critically Endangered (CR) (IUCN, 2001).

Discussion. Solms-laubachia grandiflora most closely resembles *S. linearifolia*, especially in leaf morphology and flower color, but it differs by having thickened instead of thinner, papery petioles, narrower leaves (1.5–2.3[–2.9] vs. 1.7–4.1[–5] mm wide), smaller seeds (2.4–3.6 × [1.6–]1.8–2.8[–3] mm vs. 3.5–4 × 2.5–3.5 mm), and shorter (1–3.4[–4.8] cm vs. [3–]4.5–6.5[–8] cm), less pubescent fruits. Furthermore, they differ in seed morphology, and *S. grandiflora* (Fig. 5C, D) has compound walls and smooth cells, whereas *S. linearifolia* (Fig. 5A, B) has simple walls and a minutely papillate cell surface. The molecular data from ITS (Fig. 1) show that *S. grandiflora* is clustered with *S. calcicola* and *S. sunhangania*, whereas *S. linearifolia* falls in a separate, more basal clade.

Paratype. CHINA. **Sichuan:** Xiancheng Xian, Rizhaosheng Shan, 29°6′N, 99°41′32″E, 4650 m, 18 Oct. 2005, *J. P. Yue 5120* (KUN, MO).

7. Solms-laubachia haranensis (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Desideria haranensis* Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 559. 2000, replacement name for *Ermaniopsis pumila* H. Hara, J. Jap. Bot. 49: 200. 1974, non *Solms-laubachia pumila* (Kurz) F. Dvořák, Folia Prirodoved. Fak. Univ. Purkyne Brne, Biol. 13(4): 24. 1972. TYPE: Nepal. ca. 5 mi. SW of Saldanggaon, very loose scree, 19,500 ft. [5944 m], 26 June 1952, *N. Polunin, W. R. Sykes & L. H. J. Williams 24* (holotype, BM!; isotypes, A!, BM!, E!).

Distribution. Nepal.

8. Solms-laubachia himalayensis (Cambess.) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Cheiranthus himalayensis* Cambess. in Jacquem., Voy. Inde 4: 14. 1844. *Ermania himalayensis* (Cambess.) O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 9: 1080. 1927. *Oreoblastus himalayensis* (Cambess.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. *Desideria himalayensis* (Cambess.) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 555. 2000. TYPE: [W Xizang], "in declivitate orientali jugi vulgò Kioubrung-ghauti in Tartariâ sinensi," *V. Jacquemont 1782* (holotype, P!; isotypes, K!, P!).

Distribution. China (Qinghai, Xizang), India (Himachal Pradesh, Kashmir, Punjab), Nepal (see Al-Shehbaz, 2001).

9. Solms-laubachia incana (Ovcz.) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Christolea incana* Ovcz., Sovetsk. Bot. 1941(1 & 2): 151. 1941. *Ermania incana* (Ovcz.) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 164. 1955. *Oreoblastus incanus* (Ovcz.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. *Desideria incana* (Ovcz.) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 558. 2000. TYPE: Tajikistan. Darvaz: Mt. Masar, glacier Abdul Gassan, 11,000–12,000 ft. [3353–3658 m], 23 July 1899, *V. I. Lipsky 1936* (holotype, LE!).

Distribution. Tajikistan.

10. Solms-laubachia jafrii (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Phaeonychium jafrii* Al-Shehbaz, Nordic J. Bot. 20: 160. 2000. TYPE: China. Xizang [Tibet]: hills E of Lhasa, 14,000 ft. [4267 m], on rock faces, 14 June 1942, *F. Ludlow & G. Sherriff 8714* (holotype, BM!).

Distribution. Bhutan, China (Xizang), Nepal (see Al-Shehbaz, 2000).

11. Solms-laubachia lanata Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 171. 1955. TYPE: China. Tibet [Xizang]: near Yerpa Monastery, 14,000 ft. [4267 m], Aug. 1821, *R. S. Kennedy 9* (holotype, K!).

Distribution. China (Xizang).

12. Solms-laubachia linearifolia (W. W. Sm.) O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 9: 477. 1926. Basionym: *Parrya linearifolia* W. W. Sm., Notes Roy. Bot. Gard. Edinburgh 11: 219.

1919, non *P. linearifolia* Pavlov, Vestn. Akad. Nauk Kazakhsk. S.S.R. 1: 29. 1949. TYPE: China. Yunnan: Beima Shan, Mekong–Yangtze divide, 28°20′N, 14,500 ft. [4420 m], Aug. 1914, *G. Forrest 13,235* (lectotype, designated here, E!; isotype, E!).

Solms-laubachia linearifolia var. *leiocarpa* O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 9: 477. 1926. TYPE: China. NW Yunnan: Mt. Peimashan, Mekong–Yangtze divide betw. Atuntze & Pungtzera, June 1923, *J. F. C. Rock 9304* (holotype, B!; isotypes, E!, GH!, US!).

Distribution. China (Sichuan, Xizang, Yunnan) (see Al-Shehbaz & Yang, 2001).

13. Solms-laubachia linearis (N. Busch) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Christolea linearis* N. Busch in Komarov, Fl. URSS 8: 636. 1939. *Ermania linearis* (N. Busch) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 166. 1955. *Oreoblastus linearis* (N. Busch) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. *Desideria linearis* (N. Busch) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 556. 2000. TYPE: Tajikistan. Pamir: Schugnan, Abchary, 2 Aug. 1904, *B. Fedtschenko s.n.* (holotype, LE!).

Ermania parkeri O. E. Schulz, Repert. Sp. Nov. Regni Veg. 31: 333. 1933. *Christolea parkeri* (O. E. Schulz) Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 52. 1955. *Oreoblastus parkeri* (O. E. Schulz) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 653. 1972. TYPE: [India.] Kashmir: Sonamarg, Luderwas, 13,000 ft. [3962 m], 11 Aug. 1928, *R. R. Stewart 9874A* (holotype, B!).

Ermania kashmiriana Dar & Naqshi, J. Bombay Nat. Hist. Soc. 87: 274. 1990. TYPE: [India.] Kashmir: Shalimar, Sonamarg (Sind Valley), 3900 m, 20 Aug. 1983, *G. H. Dar 7786* (holotype, KASH not seen).

Ermania kachrooi Dar & Naqshi, J. Bombay Nat. Hist. Soc. 87: 277. 1990. TYPE: [India.] Kashmir: Baltal, Sonamarg (Sind Valley), 3200 m, 2 Sep. 1982, *G. H. Dar 3934* (holotype, KASH not seen; isotypes, KASH not seen, MO!).

Distribution. China (Xinjiang, Xizang), India (Kashmir), Nepal, Tajikistan (see Al-Shehbaz & Yang, 2001).

14. Solms-laubachia mieheorum (Al-Shehbaz) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Desideria mieheorum* Al-Shehbaz, Novon 15: 1. 2005. TYPE: China. Tibet [Xizang]: Chang La, N of Sangsang, 29°41′N, 86°43′E, pioneer on frost debris, 5480–5600 m, 15 Sep. 2003, *G. & S. Miehe 03-112-21* (holotype, MO!).

Distribution. China (Xizang).

15. Solms-laubachia minor Hand.-Mazz., Anz. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 59: 246. 1922. TYPE: China. Sichuan: Mt. Holoscha, 27°48′N, betw. Yenyuen & Kwapi, 4325 m, 18 May 1914, *H. F. Handel-Mazzetti 2318* (holotype, WU!; isotypes, E!, P!, W!).

Distribution. China (Sichuan).

16. Solms-laubachia mirabilis (Pamp.) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Desideria mirabilis* Pamp., Boll. Soc. Bot. Ital. 1926: 111. 1926. *Christolea mirabilis* (Pamp.) Jafri, Fl. W. Pakistan 55: 160. 1973. TYPE: [Pakistan. Kashmir:] Karakorum, above Caracash Valley, Chisil Gilgha Pass, 5360 m, 28 June 1914, *G. Dainelli & O. Marinelli 2* (lectotype, designated here, FI not seen, FI photo at HB!).

Christolea scaposa Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 58. 1955. TYPE: [Pakistan.] Kashmir: Shaksgam Valley, 4950 m, 3 July 1926, *R. C. Clifford 7* (holotype, K!).

Christolea karakorumensis Y. H. Wu & C. H. An, Acta Phytotax. Sin. 32: 577. 1994. TYPE: China. Xinjiang: Pishan [Guma], Shenxianwan, 5250 m, 25 July 1989, *Karakorum-Kunlun Expedition 5100* (holotype, HNWP [as NWBI]!).

Desideria pamirica Suslova, Novosti Sist. Vyssh. Rast. 10: 163. 1973. *Christolea suslovaeana* Jafri, Fl. West Pakistan 55: 158. 1973, replacement names for *D. pamirica* non *Christolea pamirica* Korshinsky, Mém. Acad. Imp. Sci. Saint Pétersbourg, ser. 8, 4: 89. 1896. TYPE: Tajikistan. Pamir: above Czeczekty, near Zor, 4900 m, 10 Aug. 1970, *T. Suslova s.n.* (holotype, LE!).

Distribution. China (Xinjiang), Pakistan (Kashmir), Tajikistan (see Al-Shehbaz, 2001).

17. Solms-laubachia nepalensis (H. Hara) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Desideria nepalensis* H. Hara, J. Jap. Bot. 50: 264. 1975. TYPE: Nepal. Barum Valley, 17,700 ft. [5394 m], 26 May 1954, *L. W. Swan 71-72* (holotype, BM!).

Distribution. Nepal.

18. Solms-laubachia platycarpa (Hook. f. & Thomson) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 171. 1955. Basionym: *Parrya platycarpa* Hook. f. & Thomson, J. Proc. Linn. Soc., Bot. 5: 136. 1861, non *P. platycarpa* Rydb., Bull. Torrey Bot. Club 39: 326. 1912. TYPE: [India.] Sikkim: alpine region, 17,000 ft. [5182 m], s.d., *J. D. Hooker s.n.* (holotype, K!).

Parrya finchiana Dunn, Bull. Misc. Inform. Kew 1927: 247. 1927. TYPE: Tibet [Xizang]: along Chongphu torrent, ca. 20 mi. NE of Mt. Everest, 17,000 ft. [5182 m], 8 June 1922, *E. F. Norton 41* (holotype, K!).

Solms-laubachia orbiculata Y. Z. Lan & T. Y. Cheo, Acta Phytotax. Sin. 19: 473. 1981. TYPE: China. Xizang: Cona, 5020 m, 20 July 1975, *C. Y. Wu & S. K. Chen 75-1129* (holotype, HNWP [as HQ]!; isotype, KUN!).

Distribution. Bhutan, China (Xizang), India (Sikkim).

19. Solms-laubachia prolifera (Maxim.) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Parrya prolifera* Maxim., Fl. Tangut. 56. 1889. *Ermania prolifera* (Maxim.) O. E. Schulz, Bot. Jahrb. Syst. 66: 98. 1933. *Christolea prolifera* (Maxim.) Ovcz., Sovetsk. Bot. 1941(1 & 2): 151. 1941. *C. prolifera* (Maxim.) Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 53. 1955. *Desideria prolifera* (Maxim.) Al-Shehbaz, Ann. Missouri Bot. Gard. 87. 559. 2000. TYPE: China. Tibet [Xizang]: Kon-chun-ua, 14,500 ft., 3 July 1884, *N. M. Przewalski s.n.* (holotype, LE!; isotypes, K!, P!, PE!).

Distribution. China (Qinghai, Xizang) (see Al-Shehbaz, 2000).

20. Solms-laubachia pulcherrima Muschl. in Diels, Notes Roy. Bot. Gard. Edinburgh 5: 206. 1912. TYPE: China. NW Yunnan: E flank of Lichiang Range, 27°20′N, 12,000 ft. [3658 m], May 1906, *G. Forrest 2164* (holotype, B!; isotypes, BM!, E!, P!).

Pegaeophyton sinense (Hemsl.) Hayek & Hand.-Mazz. var. *stenophyllum* O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 9: 477. 1926. TYPE: China. Yunnan: Yangtze watershed, Prefect. Distr. of Likiang, E slope of Likiang snow range, 5300 m, 11 Aug. 1922, *J. F. C. Rock 5719* (holotype, B!; isotypes, E!, GH!, P!, US!).

Solms-laubachia pulcherrima f. *angustifolia* O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 9: 477. 1926. TYPE: China. Yunnan: Yangtze watershed, W slopes of Likiang snow range, 13,000 ft. [3962 m], 30 May–6 June 1922, *J. F. C. Rock 4277* (holotype, B!; isotypes, E!, GH!, P!, US!, W!).

Solms-laubachia pulcherrima f. *atrichophylla* Hand.-Mazz., Anz. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 62: 24. 1926. TYPE: China. Sichuan: Mt. Gonschiga, Muli monastery, Yungning toward Dschungdien, 4500 m, *H. F. Handel-Mazzetti 7503* (holotype, WU!; isotype, W!).

Parrya ciliaris Bureau & Franch., J. Bot. (Morot) 5: 20. 1891. *Solms-laubachia ciliaris* (Bureau & Franch.) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 169. 1955. TYPE: China. Tibet [Xizang]: rte. de Batang, 12 May 1890, *M. Bonvalot & H. d'Orléans s.n.* (holotype, P!).

Distribution. China (Sichuan, Xizang, Yunnan).

21. Solms-laubachia pumila (Kurz) F. Dvořák, Folia Prirodovcd. Fak. Univ. Purkyne Brne, Biol. 13(4): 24. 1972. Basionym: *Parrya pumila* Kurz, Flora 55: 285. 1872. *Vvedenskyella pumila* (Kurz) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 176. 1955. *Christolea pumila* (Kurz) Jafri, Fl. W. Pakistan 55: 157. 1973. *Desideria pumila* (Kurz) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 560. 2000. TYPE: [Kashmir.] Rupschu, 15,000–18,000 ft. [4572–5486 m], s.d., *F. Stoliczka s.n.* (holotype, CAL not seen; isotype, K!).

Ermania koelzii O. E. Schulz, Repert. Spec. Nov. Regni Veg. 31: 332. 1933. TYPE: [Pakistan] Kashmir. Rupshu, Kyensa La, 19,000 ft. [5791 m], 9 July 1931, *W. Koelz 2231* (holotype, B!).

Ermania bifaria Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad Nauk S.S.S.R. 17: 164. 1955. *Oreoblastus bifarius* (Botsch.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. TYPE: China. Xinjiang: Kuen-Lun, Humboldt Range, Ulan-Bulak, 4200 m, 30 June 1894, *W. Roborowski s.n.* (holotype, LE!).

Distribution. China (Xinjiang, Xizang), Pakistan (Kashmir).

22. Solms-laubachia retropilosa Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 171. 1955. TYPE: China. [Xizang] Tibet (as Sikkan): betw. Toutan & Lamaja, Alabosan (Ngarolak), 27 May 1893, *V. Kaschkarov s.n.* (holotype, LE!; isotype, PE!).

Solms-laubachia floribunda Y. Z. Lan & T. Y. Cheo, Acta Phytotax. Sin. 19: 475. 1981. TYPE: China. Xizang: Zogang, 5100 m, 3 July 1976, *Qinghai-Xizang Expedition 12173* (holotype, PE [as HC]!; isotypes, HNWP!, KUN!, NAS!).

Distribution. China (Sichuan, Xizang, Yunnan) (see Al-Shehbaz & Yang, 2001).

23. Solms-laubachia stewartii (T. Anderson) J. P. Yue, Al-Shehbaz & H. Sun, comb. nov. Basionym: *Cheiranthus stewartii* T. Anderson in J. D. Hooker, Fl. Brit. India 1: 132. 1872. *Ermania stewartii* (T. Anderson) O. E. Schulz, Bot. Jahrb. Syst. 66: 98. 1933. *Christolea stewartii* (T. Anderson) Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 53. 1955. *Oreoblastus stewartii* (T. Anderson) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 653. 1972. *Desideria stewartii* (T. Anderson) Al-Shehbaz, Ann. Missouri Bot. Gard. 87: 556. 2000. TYPE: [India] Kashmir. Ladak, 15,000–16,500 ft. [4572–5029 m], s.d., *J. L. Stewart s.n.* (holotype, K!; isotype, E!).

Distribution. China (Xizang), India (Himachal Pradesh, Punjab, Kashmir) (see Al-Shehbaz & Yang, 2001).

24. Solms-laubachia sunhangiana J. P. Yue & Al-Shehbaz, sp. nov. TYPE: China. Sichuan: Jiulong Xian, Tanggu Xiang, NW of city of Jiulong, Wuxu

Hai (Wuxu Lake), 29°9′11″N, 101°24′25″E, *Abies*, *Picea*, *Quercus* forest with *Rhododendron* understory & open, grazed, dry, and boggy meadows, crevices of rocks, 4175 m, 22 June 2005, *D. E. Boufford, J. H. Chen, S. L. Kelley, J. Li, R. H. Ree, H. Sun, J. P. Yue & Y. H. Zhang 33464* (holotype, KUN!; isotypes, A!, MO!).

Herba perennis pulvinata, 1–3 cm alta. Folia basalia rosulata, linearia vel lineari-oblanceolata, 0.8–2.5 cm × 1–2.5 mm, sparse pubescentia, integra; petiolis 3.5–5.5 mm longis, incrassatis; folia caulina nulla. Pedicelli fructiferi solitarii, 0.8–1.2 cm longi. Sepala libera, oblonga, 2–3 × 1–1.2 mm, sparse pilosa; petala ca. 6 mm longa; ovula 8 ad 12. Fructus lineares vel lineari-lanceolati, 1.6–2.5 cm × 2.8–3.2 mm, pubescentes. Semina ovato-oblonga, ca. 2.5 × 1.5 mm.

Herbs perennial, pulvinate, 1–3 cm tall; caudex covered with petioles and intact leaves of previous years. Basal leaves rosulate; blade linear to linear-oblanceolate, 0.8–2.5 cm × 1–2.5 mm, grooved proximally, sparsely pubescent, not ciliate, base attenuate, margin entire; petioles 3.5–5.5 mm, thickened; cauline leaves absent. Fruiting pedicels solitary from basal rosette, 0.8–1.2 cm long. Sepals free, oblong, 2–3 × 1–1.2 mm, sparsely pilose; petals ca. 6 mm; anthers ca. 1 mm; ovules 8 to 12 per ovary. Fruit linear-lanceolate to linear, 1.6–2.5 cm × 2.8–3.2 mm; valves pubescent, obscurely reticulate veined; septum complete; style 0.6–1 mm; stigma strongly 2-lobed. Seeds ovate-oblong, ca. 2.5 × 1.5 mm.

Distribution. Narrowly endemic to Jiulong Xian in Sichuan Province, China.

IUCN Red List category. Critically Endangered (CR) (IUCN, 2001).

Discussion. Solms-laubachia sunhangiana is named in honor of Sun Hang, deputy director of the Kunming Institute of Botany, for his continuous support of fieldwork on the genus. This novelty is easily distinguished from the remaining species of the genus with entire leaves by having much smaller flowers (sepals 2–3 mm vs. [5.5–]5–8[–11] mm long) and narrower fruits (2–3 mm vs. [4–]5–18 mm wide). It is closely related to *S. xerophyta*, which it resembles by having persistent, linear, narrow (less than 3 mm wide) leaves grooved proximally. Such leaves are also found in *S. zhongdianensis*, a species unique in the genus by its thinner, papery petioles instead of thickened ones.

25. Solms-laubachia xerophyta (W. W. Sm.) H. F. Comber, Notes Roy. Bot. Gard. Edinburgh 18: 249. 1934. Basionym: *Parrya xerophyta* W. W. Sm., Notes Roy. Bot. Gard. Edinburgh 12: 217. 1920. TYPE: China. Yunnan: NE of Chungtien, 27°55′N, July 1918, *G. Forrest 16444* (holotype, E!; isotypes, E!, K!, P!, W!).

Distribution. China (Sichuan, Yunnan) (see Al-Shehbaz & Yang, 2001).

26. Solms-laubachia zhongdianensis J. P. Yue, Al-Shehbaz & H. Sun, Ann. Bot. Fenn. 42: 156. 2005. TYPE: China. Yunnan: Shangri-la Co., Mt. Shika, scree, sandy areas, 27°47′N, 99°35′E, ca. 4450 m, 27 Sep. 2001, *J. P. Yue 154* (holotype, KUN!; isotype, MO!).

Distribution. China (Yunnan).

Conservation Status

The four new species described above (*S. angustifolia*, *S. calcicola*, *S. grandiflora*, and *S. sunhangiana*) are known from only their type specimens or a few additional collections. Following the IUCN guidelines (IUCN, 2001), and because all four have very narrow distribution ranges, our preliminary conservation assessment would indicate that they are Critically Endangered (CR).

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CHROMOSOME NUMBERS IN VERONICEAE (PLANTAGINACEAE): REVIEW AND SEVERAL NEW COUNTS[1]

D. C. Albach,[2] *M. M. Martínez-Ortega,*[3] *L. Delgado,*[3] *H. Weiss-Schneeweiss,*[4] *F. Özgökce,*[5] *and M. A. Fischer*[4]

ABSTRACT

Chromosomal evolution in *Veronica* L. and related genera (*Wulfenia* Jacq., *Wulfeniopsis* D. Y. Hong, *Paederota* L., *Lagotis* Gaertn., *Picrorhiza* Royle ex Benth., and *Veronicastrum* Heist. ex Fabr.; Veroniceae, Plantaginaceae; formerly Scrophulariaceae) is presented. To this end, we conducted an extensive literature survey of more than 400 publications covering ca. 300 out of 500 species in the tribe. We also report 44 new chromosome counts. Chromosome numbers of *Veronica hispidula* Boiss. & Huet. ($2n = 18, 36$) and *V. reuteriana* Boiss. ($2n = 28, 42$) are reported for the first time, and both species exhibit intraspecific ploidy level variation. Other new counts confirm chromosome numbers reported previously. The evolution of chromosome numbers in Veroniceae is discussed in light of recent results from DNA-based phylogenetic analyses. Most of the subgenera of *Veronica* exhibit only one single basic number, i.e., $x = 6, 7, 8, 9, 12, 17$, or 20/21. In this genus, the putative ancestral base number of 9 has been reduced several times to 8 and 7, respectively (aneuploidy/dysploidy), often associated with transition to annual life history. In contrast, no unambiguous increase of chromosome base number has been inferred. A table that includes all species of Veroniceae and, if known, their chromosome number and full sectional and subsectional classification in *Veronica* is provided. For this purpose, three new combinations have been introduced (*Veronica* sect. *Acinifolia* (Römpp) Albach, *Veronica* sect. *Glandulosae* (Römpp) Albach, and *Veronica* subsect. *Cochlidiosperma* (Rchb.) Albach).

Key words: Annual habit, chromosome numbers, dysploidy, Plantaginaceae, polyploidy, *Veronica*, Veroniceae.

The tribe Veroniceae within the Plantaginaceae (sensu Angiosperm Phylogeny Group, 2003; formerly part of Scrophulariaceae) comprises about 500 species distributed mainly in temperate regions of the Northern Hemisphere and Australasia. By far, the largest genus in the tribe is *Veronica* L., with ca. 450

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species, which has recently been recircumscribed to include (again) the Australasian species of the Hebe complex (Albach et al., 2004b; Garnock-Jones et al., 2007). Growing information on phylogenetic relationships in the tribe (e.g., Albach & Chase, 2001, 2004; Albach et al., 2004a, c) has highlighted the need to summarize available information for characters important for the evolution of the tribe and to point out critical taxa for which this character has not yet been investigated. One of these characters is chromosome number. Since the first publication of chromosome numbers in *Veronica* by Heitz (1926), many authors have published chromosome numbers, including some extensive regional surveys (e.g., Iceland, Löve & Löve, 1956; Belarus, Dzhus & Dmitrieva, 2001) and other extensive surveys of smaller groups within *Veronica* (e.g., subsection *Acinifolia* (Römpp) Stroh; Fischer, 1972). Albach et al. (2004a) gave the first overview of the evolution of chromosome numbers in Veroniceae. The principal result was that $x = 9$ is the ancestral chromosome number in *Veronica*, with just one reduction to $x = 8$ but four independent reductions to $x = 7$ (once from $x = 8$, but three times from $x = 9$) (Albach et al., 2004a). Dysploidy is, in most cases, associated with a transition to annual life history (Albach et al., 2004a). Here, we investigate the evolution of chromosome numbers of Veroniceae in more detail and highlight those species or populations that need further investigation. Evolutionary relationships detected in major contributions to floras (e.g., Turkey and Iran, Fischer, 1978, 1981; China, Hong & Fischer, 1998; Spain, Martínez-Ortega et al., in press) combined with results from molecular systematic analyses (e.g., Albach et al., 2004a) form the basis of the discussion.

The present contribution is based on previously published chromosome numbers, but also includes 44 new reports of chromosome numbers. This review presents a first attempt to survey all chromosome numbers worldwide for the entire tribe Veroniceae, and although we are aware that our list is not yet complete, we hope that this review will allow some evolutionary conclusions and also stimulate chromosome studies of those species that are the most interesting but not yet counted. Finally, we want to strongly emphasize the great need for good quality figures accompanying the publication of any new chromosome numbers and the necessity to provide voucher information for each count.

Materials and Methods

We have found more than 400 publications including chromosome numbers in Veroniceae representing more than 2600 populations. Several comprehensive reviews of chromosome numbers for regional floras (e.g., the former Soviet Union, Agapova et al., 1993; Austria, Dobeš & Vitek, 2000) as well as worldwide (e.g., Goldblatt & Johnson, 2006) do exist and greatly facilitated this work. Concomitantly, frequent errors have been encountered in these surveys. Whenever feasible, we checked the original source, and eventually we checked ca. 350 original publications (including ca. 2500 studied populations within the Veroniceae).

Here, we use the supraspecific classification of Albach et al. (2004b) and the species in Veroniceae currently accepted by us (Albach, Martínez-Ortega & Fischer, unpublished data). Author names of taxa are given in Appendix 1. Original chromosome counts were conducted mostly using seeds collected in the field and germinated in the laboratory. For *Veronica bellidioides* L., actively growing root tips of plants cultivated in the Botanical Garden of the University of Vienna (Austria) were used. Root tips were pretreated with 0.002 M 8-hydroxyquinoline for 2 hr. at room temperature and 1.5 hr. at 4 C, and fixed in 3 ethanol:1 acetic acid for 24 hr. at room temperature and stored at −20° C or 4°C until use. For *V. persica* Poir. as well as other taxa studied by LD and MMMO, flower buds were collected in the field and fixed in modified Carnoy's solution (4 chloroform:3 absolute ethanol:1 glacial acetic acid), transferred to 70% ethanol, and stored at 4 C until use. For chromosome counts by HWS and DCA, material was hydrolyzed in 5N HCl for 20 min., washed, and Feulgen-stained in Schiff's reagent (Fukui & Nakayama, 1996) for 1–1.5 hr. in the dark. Material analyzed by MMMO and LD was stained in 2% acetic orcein (La Cour, 1954). All squash preparations were made in a drop of 45% acetic acid. Chromosome numbers were analyzed under light microscopy (Carl Zeiss AG, Oberkochen, Germany) by HWS and DCA or Nikon Optiphot (Nikon Corp., Tokyo, Japan) by MMMO and LD and documented with black-and-white photography (Fig. 1). For each individual, several cells (a minimum of five) with well-spread chromosomes were used for chromosome number determination. Chromosome counts by MAF followed the methods given in Fischer (1975a). Vouchers from DCA and MAF were deposited in the herbarium of the University of Vienna (WU) and those from MMMO and LD at the University of Salamanca (SALA).

In an electronic appendix (available at <http://www.spezbot.fb10.uni-mainz.de/home_e/img/albach_appendix.xls>), we have summarized ca. 2600 chromosome counts from ca. 400 publications. Both haploid and diploid counts were considered. The original plant name under which the count was published as well as the currently accepted name are given.

To test whether chromosome number (n, $2n$), chromosome base number (x), and ploidy levels significantly differ between annuals and perennials, the Mann-Whitney U test has been used. To avoid an overly large influence from the large radiation of Australasian species in the *Veronica*, which are likely of hexaploid origin with respect to other *Veronica*, statistical comparisons were conducted with and without Australasian species as well as those species with $x = 17$ (*Veronicastrum* Heist. ex Fabr., *Picrorhiza* Royle ex Benth., *Veronica* subg. *Pseudolysimachium* (Opiz) Buchenau).

Results and Discussion

Forty-four chromosome counts reported here for the first time are summarized in Table 1, and for 13 significant reports, photographs are presented (Fig. 1). Currently, chromosome numbers for 330 of 509 species (65%) in Veroniceae have been published (Appendix 1, see electronic appendix for a comprehensive listing at <http://www.spezbot.fb10.uni-mainz.de/home_e/img/albach_appendix.xls>). However, some groups are well known (e.g., *Veronica* subg. *Cochlidiosperma* (Rchb.) M. M. Mart. Ort. & Albach and *Veronica* subg. *Synthyris* (Benth.) M. M. Mart. Ort., Albach & M. A. Fisch.—all known species have been counted), whereas other groups include many species for which no count is available. Most notably, chromosome numbers of less than one fourth (23%) of the species of *Lagotis* Gaertn. and only about one third (32%) of the species of *Veronica* subg. *Stenocarpon* (Boriss.) M. M. Mart. Ort., Albach & M. A. Fisch are known. Chromosome base numbers (Table 2) and ploidy levels (Table 3) have been summarized, arranged by subgenus in *Veronica*. Albach et al. (2004a) discussed the ancestral chromosome number in Veroniceae, which is inferred to be $x = 9$, although $x = 5$ is also possible (Lepper, 1964; Albach & Chase, 2004). They further inferred a single origin of $x = 8$ within *Veronica* (the $x = 8$ clade). However, a more detailed analysis shows that independent origins of $x = 8$ occurred in several instances independently (Fig. 2; see below). Albach et al. (2004a) also reported four independent changes to $x = 7$ (Fig. 2), which now needs to be revised to six changes (two from $x = 9$, three from $x = 8$, one ambiguous optimization) because of overlooked numbers in *V. densiflora* Ledeb. (*Veronica* subg. *Stenocarpon*) and *V. magna* M. A. Fisch. (*Veronica* subg. *Chamaedrys* (W. D. J. Koch) Buchenau). No unambiguous chromosome number increases between $x = 7$, 8, and 9 are inferred (Albach et al., 2004a; and updated by information presented here).

The evolution of an annual life history in *Veronica* has been studied in various aspects in recent years. Molecular phylogenetic analyses have shown that an annual life history arose several times independently (Albach et al., 2004c). Analyzing genome size in *Veronica* has demonstrated that a low genome size is correlated with selfing rather than with annual life history (Albach & Greilhuber, 2004) as previously suggested (Bennett, 1972). A higher DNA substitution rate, however, is not correlated with selfing, but rather with an annual life history in *Veronica* (Albach & Müller, in prep.), supporting more general results for all angiosperms (Bousquet et al., 1992). Therefore, it seems appropriate to analyze whether chromosome numbers differ between annuals and perennials. Several studies (e.g., Levin, 2002) have suggested a reduction in chromosome number associated with the shift from perennial to annual life history. Standard statistics are not as powerful as statistical methods incorporating phylogenetic evidence due to the confounding effect of shared evolutionary history on the results (Harvey & Rambaut, 1998). However, a fully resolved phylogeny for all species of Veroniceae for which chromosome numbers are available does not exist and is not even possible to generate due to the reticulate history especially of polyploid taxa (e.g., Albach, 2007). The results of the statistical analysis nevertheless appear robust in showing annual species to have a significantly lower chromosome base number but not a lower ploidy level. The inclusion of Australasian species of *Veronica* and those species with $x = 17$ (*Veronicastrum*, *Picrorhiza*, *Veronica* subg. *Pseudolysimachium*) in an analysis has the effect that annual species have a significantly lower chromosome number and ploidy level. Without those perennial species, annual species have on average a ploidy level of 3.3, whereas perennial species have a ploidy level of 3.4, which is not significantly different ($P = 0.85$). Chromosome base numbers, however, differ significantly, with a lower number found in annuals ($P < 0.01$). We could not check whether this is an indirect effect of a correlation of selfing with a lower chromosome base number, as is the case in the evolution of genome size (Albach & Greilhuber, 2004), due to the paucity of information on breeding systems in the Veroniceae. Both reduction in chromosome number and selfing may reduce recombination rates and, therefore, it appears likely that both are selected for instances in which a low recombination rate is advantageous. The chromosome number is the product of ploidy level and chromosome base number, and its distribution is not significantly different ($P = 0.19$) within the Veroniceae.

Veroniceae Excluding *Veronica*

Veroniceae in the circumscription of Albach et al. (2004b) comprise nine genera, including two mono-

Table 1. Information on chromosome counts in *Veronica* reported here for the first time. Counts performed by DCA, HWS, LD, MAF, and MMMO.

Species	Chromosome number	Counted by	Origin	Voucher
V. *alpina* L.	$2n = 18$	LD, MMMO	Spain. Huesca: Torla, Bujaruelo, N face of Pico Gabieto	*M. M. Martínez-Ortega 300* (SALA)
V. *alpina*	$2n = 18$	LD, MMMO	Spain. Huesca: Benasque, La Renclusa, 7 Aug. 1997	*M. M. Martínez-Ortega s.n.* (SALA)
V. *anagalloides* subsp. *heureka* M. A. Fisch.	$2n = 18$	HWS, DCA	Georgia. Greater Caucasus: Cross Pass	*D. C. Albach 299* (WU)
V. *aphylla* L.	$n = 9$, $2n = 18$	LD, MMMO	Spain. Navarra: Isaba, Portillo de Arrasarguiat	*L. Delgado 337* (SALA 110613)
V. *arguteserrata* Regel & Schmalh.	$2n = 42$, Fig. 1A	HWS, DCA	Turkey. Bitlis: Murat valley betw. Hamur and Tutak	*D. C. Albach 685* (WU)
V. *bellidioides* L.	$2n = 36$	HWS, DCA	Bulgaria. Mt. Pirin	*D. C. Albach 551* (WU)
V. *bellidioides*	$2n = 36$	HWS	Spain. Pyrenees	*P. Schönsweiter 148/21* (WU)
V. *bombycina* Boiss. & Kotschy subsp. *bombycina*	$2n = 16$	MAF	Cult. Bot. Gard. Vienna, 13 May 1969	*M. A. Fischer s.n.* (WU)
V. *bozakmanii* M. A. Fisch.	$2n = 28$	HWS, DCA	Turkey. Bitlis: Kucuksu	*D. C. Albach 611* (WU)
V. *bozakmanii*	$2n = 28$, Fig. 1B	HWS, DCA	Turkey. Bitlis: W of Kuzgunkegan Pass	*D. C. Albach 667* (WU)
V. *campylopoda* Boiss.	$2n = 42$, Fig. 1C	DCA	Turkey. Van: W of Güzeldere Pass	*D. C. Albach 653* (WU)
V. *campylopoda*	$2n = 56$, Fig. 1D	DCA	Turkey. Van: Catak (cultivated at Technical University of Denmark, Lyngby)	*Jensen IOK-6/2003* (WU)
V. *chamaedrys* L. subsp. *chamaedrys*	$2n = 32$	LD, MMMO	Spain. Oviedo: Quirós, toward Pico Gamoniteiro	*L. Delgado 768 & E. Rico* (SALA 110656)
V. *chamaedrys* subsp. *chamaedrys*	$2n = 32$	LD, MMMO	Spain. Teruel: from Fontanete to Valdelinares	*E. Rico 7078* (SALA 110655)
V. *ciliata* subsp. *cephaloides* (Pennell) D. Y. Hong	$2n = 16$, Fig. 1K	HWS, DCA	China. Xizang (Tibet)	*G. Miehe 98-16717* (GOET)
V. *cymbalaria* Bodard	$2n = 36$	MAF	Greece. Crete, Omalos: Xyloskalon, 6 May 1971	*H. Malicky s.n.* (WU)
V. *cymbalaria*	$2n = 36$	MAF	Greece. Crete, Ida mtns.: near Ideon Antron cave, 9 May 1971	*H. Malicky s.n.* (WU)
V. *cymbalaria*	$2n = 54$	MAF	Greece. Ionian Islands, Kefallinia: area betw. Sami & Dhihalia, 11 Apr. 1974	*M. & G. Fischer s.n.* (WU)
V. *cymbalaria*	$2n = 54$	MAF	Greece. Ionian Islands, Kefallinia: Mt. Rudi, 13 Apr. 1974	*M. & G. Fischer s.n.* (WU)
V. *cymbalaria*	$2n = 54$	MAF	Greece. Ionian Islands, Kefallinia: area betw. Sami & Dhihalia, 14 Apr. 1974	*M. & G. Fischer s.n.* (WU)
V. *filiformis* Sm.	$2n = 14$	Tanja Lessel, DCA	Austria, Graz: Schubertstrasse, in front of Botanical Garden	*D. C. Albach 857* (MJG)
V. *fruticans* subsp. *cantabrica* M. Laínz	$n = 8$, $2n = 16$	LD, MMMO	Spain. Cantabria: Hermandad de Campoo de Suso, base of Pico Tres Mares, 11 July 1996	*M. M. Martínez-Ortega s.n.* (SALA)
V. *fruticans* subsp. *cantabrica*	$2n = 16$	LD, MMMO	Spain. Segovia: El Cardoso de la Sierra, Pico del Lobo, 2 July 1996	*M. M. Martínez-Ortega s.n.* (SALA)
V. *fruticans* subsp. *cantabrica*	$2n = 16$	LD, MMMO	Spain. La Rioja: Canales de la Sierra, Pico Gatón	*M. M. Martínez-Ortega s.n.* (SALA)

Table 1. Continued.

Species	Chromosome number	Counted by	Origin	Voucher
V. fruticans subsp. *cantabrica*	$2n = 16$	LD, MMMO	Spain. Ávila: Solana de Ávila, Lagunas de El Trampal, N slope	*E. Rico 7105* (SALA 110610)
V. fruticans subsp. *cantabrica*	$2n = 16$	LD, MMMO	Spain. Lérida: Pla de Beret	*L. Delgado 344 & I. Soriano* (SALA 110609)
V. gentianoides Vahl	$2n =$ ca. 72	DCA	Georgia. Greater Caucasus: W of Truso Gorge	*D. C. Albach 350* (WU)
V. gentianoides	$2n = 36$, Fig. 1I	HWS, DCA	Georgia. Greater Caucasus: meadows above Zaminda Sameba	*D. C. Albach 341* (WU)
V. gentianoides	$2n =$ ca. 72	DCA	Georgia. Greater Caucasus: beech forest below Zaminda Sameba	*D. C. Albach 318* (WU)
V. gentianoides	$2n = 46$–48, Fig. 1H	DCA	Georgia. Greater Caucasus: below Dzuta	*D. C. Albach 333* (WU)
V. hispidula Boiss. & Huet.	$2n = 18$ (5 seedlings), Fig. 1E; $2n = 36$ (1 seedling) Fig. 1G	HWS, DCA	Turkey. Van: Degitmenköy	*D. C. Albach 635* (WU)
V. micrantha Hoffmans. & Link	$n = 8$, $2n = 16$, Fig. 1M	LD, MMMO	Spain. Salamanca: Peñaparda, Mostejal brook	*L. Delgado 34, Y. Giráldez & E. Rico 16-VI-1998* (SALA 110657)
V. nummularia Gouan	$2n = 16$, Fig. 1J	LD, MMMO	Spain. Huesca: Ansó, Collado de Petrachema, N side	*L. Delgado 332 & I. Soriano 25-VI-2000* (SALA 110612)
V. peduncularis M. Bieb.	$2n = 16$–18	DCA	Georgia. Greater Caucasus: Kazbegi	*D. C. Albach 325* (WU)
V. persica Poir.	$2n = 28$	HWS	Azerbaijan. Talysh: forest reserve Qirkan qorugu, SW of Lankaran	*G. Schneeweiss Geo01 132/28* (WU)
V. ponae Gouan	$2n = 54$	LD, MMMO	Spain. Huesca: Panticosa, toward lakes	*M. Santos Vicente 455, L. P. Gavilán Iglesias & L. Mª. Muñoz Centeno* (SALA 110608)
V. prostrata L.	$2n = 16$	LD, MMMO	Czech Republic. Beroun	*M. M. Martínez-Ortega 1331* (SALA)
V. reuteriana Boiss.	$2n = 28$, Fig. 1L	HWS, DCA	Turkey. Van: Catak	*D. C. Albach 691* (WU)
V. reuteriana	$2n = 42$, Fig. 1F	HWS, DCA	Turkey. Bitlis: S of Tutak	*D. C. Albach 676* (WU)
V. scheereri (J. P. Brandt) Holub	$2n = 32$	LD, MMMO	Spain. Huesca: Ansó, on the way to Collado de Petrachema	*L. Delgado 328 & I. Soriano* (SALA 110643)
V. scheereri	$2n = 32$	LD, MMMO	Germany. Baden-Württemberg: Schwäbische Alb	*M. M. Martínez-Ortega 1093* (SALA)
V. scutellata L.	$2n = 18$	LD, MMMO	Spain. Salamanca: Fuenteguinaldo, meadows of El Potril, banks of Río Agueda, 17 June 1996	*M. M. Martínez-Ortega s.n. & E. Rico* (SALA)
V. sp. indet., aff. *beccabungoides*	$2n = 36$	HWS, DCA	Georgia. Greater Caucasus: Kazbegi	*D. C. Albach 329* (WU)
V. trichadena Jord. & Fourr.	$2n = 36$	MAF	Turkey. Mugla: Sakar pass, ca. 15 km S of Mugla, 28 Mar. 1978	*G. & M. A. Fischer s.n.* (WU)

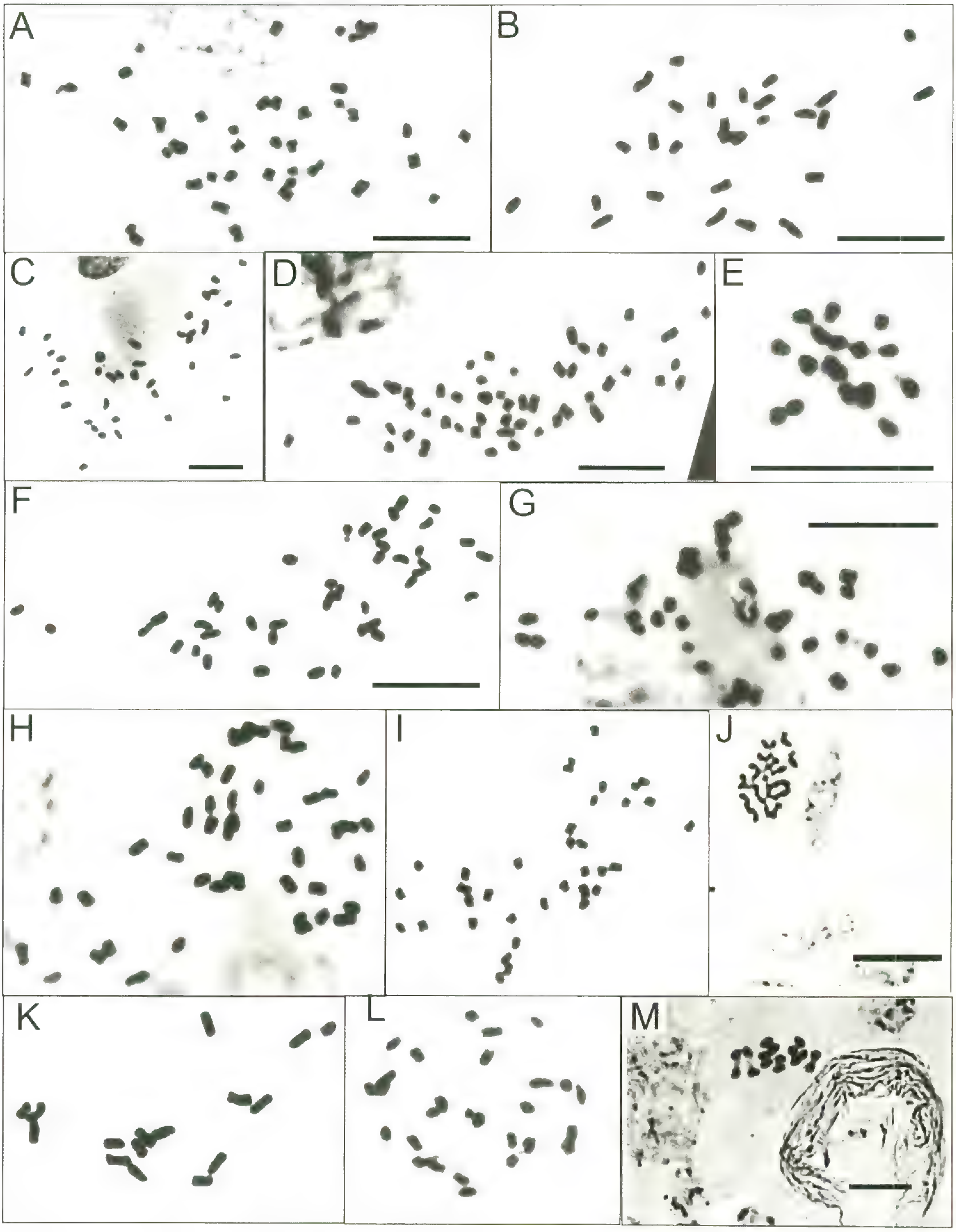

Figure 1. Photos of chromosome counts. Scale bars = 10 μm. —A. *Veronica arguteserrata* (*Albach 685*, WU). —B. *V. bozakmanii* (*Albach 667*, WU). —C. *V. campylopoda* (*Albach 653*, WU). —D. *V. campylopoda* (*Jensen IOK-6/2003*, WU). —E. *V. hispidula* (*Albach 635*, WU). —F. *V. reuteriana* (*Albach 676*, WU). -G. *V. hispidula* (*Albach 635*, WU). —H. *V. gentianoides* (*Albach 333*, WU). —I. *V. gentianoides* (*Albach 341*, WU). —J. *V. nummularia* (*Delgado 332*, SALA). —K. *V. ciliata* subsp. *cephaloides* (*Miehe 98-16717*, GOET). —L. *V. reuteriana* (*Albach 691*, WU). —M. *V. micrantha* (*Delgado 34*, SALA).

Table 2. Chromosome base number for species arranged according to major clades in Veroniceae. Ancestral character state for each clade is underlined, based on information given in Albach et al. (2004a) and Wagstaff and Garnock-Jones (1998).

	$x = 7$	$x = 8$	$x = 9$	$x = 17$	$x = 20$	$x = 21$	$x =$ other	Unknown
Veroniceae excluding *Veronica*		2	6	6			7	40
Veronica subgen. *Veronica*	2	3	19				1	20
Subg. *Beccabunga*	8	2	13					10
Subg. *Pseudolysimachium*				18				11
Subg. *Synthyris*							18	
Subg. *Cochlidiosperma*			10				1	1
Subg. *Pellidosperma*	1	1	2					3
Subg. *Stenocarpon*		11						22
Subg. *Pocilla*	14							14
Subg. *Pentasepalae*		33						37
Subg. *Chamaedrys*	1	11						1
Sect. *Derwentia*				1	4	4	11	3
Sect. *Hebe*					69	46	3	16
Other species of *Veronica*		1					2	—
Total (out of 509 species)	26	64	50	25	73	50	43	178

typic ones for which no chromosome numbers are known (*Scrofella* Maxim. from China, *Kashmiria* D. Y. Hong from the Himalayas).

Lagotis is the least studied group in Veroniceae despite its wide occurrence from eastern Turkey to Alaska. Although 35 chromosome numbers are available, only seven of 31 species in the genus have been studied. All reported chromosome numbers are based on $x = 11$, except for $2n = 54$ for *L. brevituba* Maxim. (Huang et al., 1996). Unfortunately, no figure is included in the latter publication and, therefore, this number requires confirmation. *Lagotis stolonifera* (K. Koch) Maxim. (two counts), *L. takedana* Miyabe & Tatew. (one count), *L. cashmeriana* (Royle) Rupr. (three counts), and *L. glauca* Gaertn. (five counts) are diploids ($2n = 2x = 22$), whereas *L. integrifolia* (Willd.) Schischk. (three counts) is tetraploid ($2n = 4x = 44$). *Lagotis minor* (Willd.) Standl. has been studied intensively (16 counts), with more than half (nine counts) being tetraploid ($2n = 2x = 44$) and the rest diploid ($2n = 2x = 22$). Most of the counts for *L. minor* are from Russia, with only one diploid count from Canada. The taxonomic concept of *L. minor* and related species is complex, and confusion with *L.*

Table 3. Occurrence of polyploidy in various taxa of Veroniceae and among annual and perennial species of the tribe. In the line labeled "Perennial species," note that Australasian taxa and those with $x = 17$ have been excluded.

	$2x$	$4x$	$6x$	$8x$	$10x$	$12x$	$16x/18x$
Non-*Veronica* Veroniceae	10	10	1	1			1
Subg. *Veronica*	14	6	6	1			
Subg. *Beccabunga*	13	14	5	1	1		
Subg. *Pseudolysimachium*		16		9			
Subg. *Synthyris*	16	5					
Subg. *Cochlidiosperma*	7	3	2				
Subg. *Pellidosperma*	4						
Subg. *Stenocarpon*	10	1					
Subg. *Pocilla*	6	7	3	1			
Subg. *Pentasepalae*	22	7	5	6	2		
Subg. *Chamaedrys*	10	2	1				
Sect. *Derwentia*			15			5	
Sect. *Hebe*			87			26	15
Others	2	1					
Total	117	68	118	8	4	26	16
Annual species	30	16	8	1			
Perennial species	86	35	15	8	3		
All perennial species	86	56	117	18	3	31	16

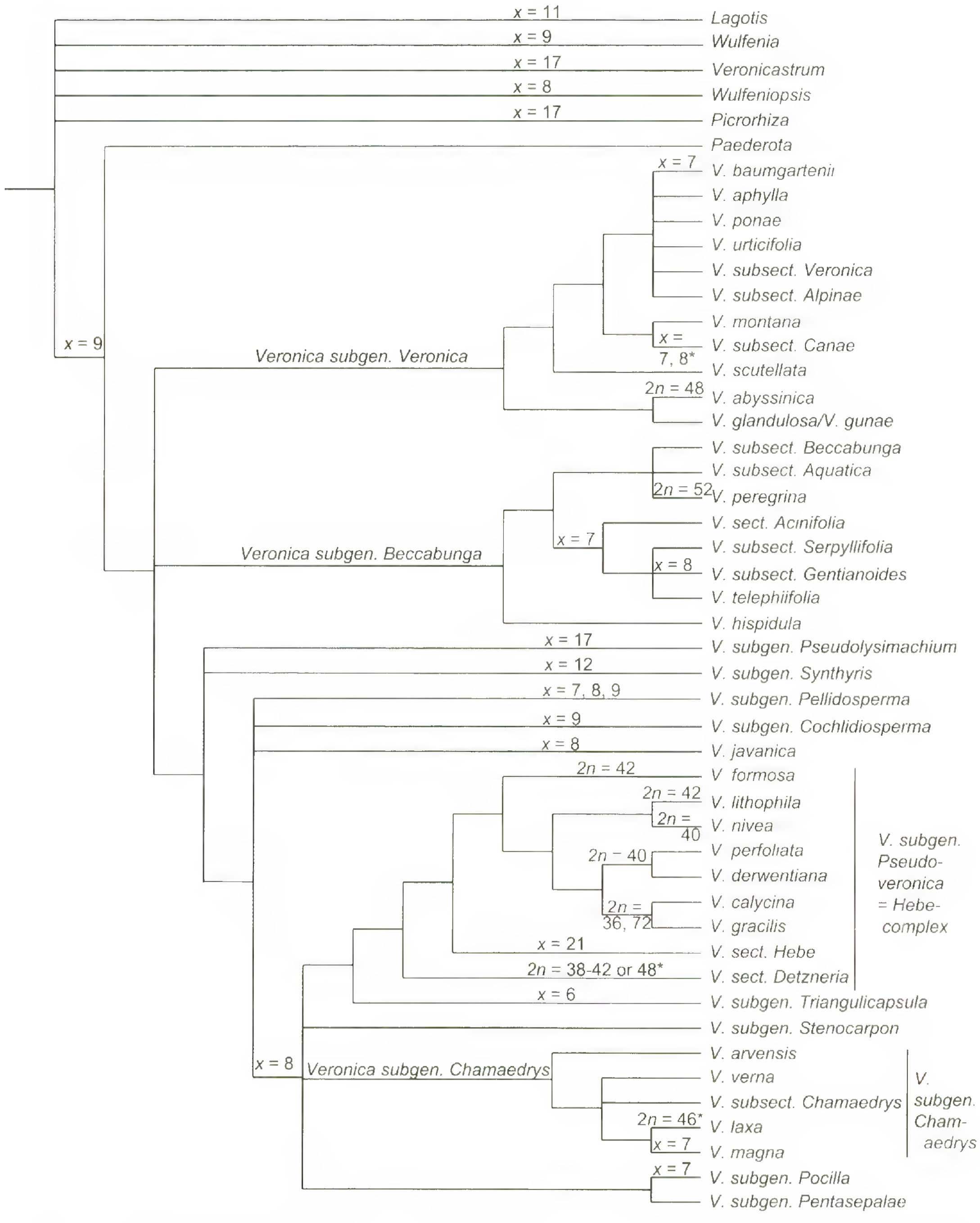

Figure 2. Cladogram showing the relationships of Veroniceae based on information from Albach et al. (2004a, c; 2005a, b; Albach, unpublished data). Asterisks mark counts that are considered dubious (see text for details).

glauca in the east and *L. integrifolia* in the south of the distribution is likely (Hultén, 1930). Unfortunately, voucher specimens for many counts are not cited clearly. A detailed analysis using morphological, karyological, and molecular methods would, therefore, be necessary to disentangle the taxonomic problems around *L. minor*.

Wulfenia Jacq. (from the southeastern Alps, western Balkan Peninsula, and southern Turkey) and *Paederota* L. (from the southeastern Alps) have $x = 9$, with *Wulfenia* being diploid ($2n = 2x = 18$; *W. carinthiaca* Jacq., *W. blecicii* Lakušić, *W. orientalis* Boiss., and *W. baldacii* Degen with the first counted 11 times, and the latter three counted just once each) and *Paederota*

being tetraploid ($2n = 4x = 36$), although a single hexaploid plant of *P. lutea* Scop. ($2n = 6x = 54$) was found by Fischer (1969).

Wulfeniopsis D. Y. Hong (from Afghanistan to Nepal) includes two species that were both reported mostly as diploids ($2n = 2x = 16$) based on $x = 8$ (*W. amherstiana* (Benth.) D. Y. Hong, 11 counts; *W. nepalensis* (T. Yamaz.) D. Y. Hong, 1 count), with only one plant being tetraploid ($2n = 4x = 32$; *W. amherstiana*). *Veronicastrum* (from eastern Asia) and *Picrorhiza* (from the Himalayas) have been demonstrated to be tetraploid hybrids of *Wulfeniopsis* with *Wulfenia* and the common progenitor of *Veronica* plus *Paederota*, respectively (Albach & Chase, 2004). Therefore, $2x_{\mathrm{Pic}}$ and $2x_{\mathrm{Vst}}$ are $4x$ with respect to the rest of the tribe. Chromosome numbers are only available for one of three species of *Picrorhiza* (*P. kurrooa* Royle ex Benth, single count; $2n = 2x_{\mathrm{Pic}} = 34$) and six of 18 species of *Veronicastrum*, with *V. sibiricum* (L.) Pennell (7 counts), *V. virginicum* Farw. (3 counts), *V. brunonianum* (Benth.) D. Y. Hong, and *V. japonicum* (Nakai) T. Yamaz. (both counted once) being diploid ($2n = 2x_{\mathrm{Vst}} = 34$), *V. villosulum* (Miq.) T. Yamaz. tetraploid ($2n = 4x_{\mathrm{Vst}} = 68$; one count), and *V. liukiuense* (Ohwi) T. Yamaz. octoploid ($2n = 8x_{\mathrm{Vst}} = 136$; one count) based on $x = 17$, which is the sum of the chromosome numbers of their inferred parents ($x_{\mathrm{Pic}}/x_{\mathrm{Vst}} = 8 + 9$).

Veronica s.l., according to Albach et al. (2004b) and Garnock-Jones et al. (2007), consists of 12 subgenera.

I. *VERONICA* SUBG. *VERONICA*

Veronica subg. *Veronica* includes 45 species with chromosome numbers available for 25 (Appendix 1). Molecular systematic analyses (Albach & Chase, 2001; Albach et al., 2004a, b) have identified an early branching clade that includes the African species of *Veronica*, as well as *V. scutellata* L., and a group of montane to subalpine Chinese–Himalayan species plus *V. montana* L. as consecutive sisters to a crown group of montane to subalpine species from the Northern Hemisphere including the type species *V. officinalis* L. (within subsection *Veronica*, Fig. 2). African species are characterized by high chromosome numbers. The two closely related species *V. glandulosa* Hochst. ex Benth. and *V. gunae* Schweinf. ex Engl. are both hexaploid ($2n = 6x = 54$) based on $x = 9$ (the most common base number in the group; two counts each), although only the extreme western populations of *V. glandulosa* from Cameroon, corresponding to subspecies *mannii* (Hook. f.) Elenevsky, have been counted. The annual *V. abyssinica* Fresen. also seems to be hexaploid ($2n = 6x = 48$; one count) based on $x = 8$; however, the number is based on only one report. Clearly, more analyses for this species are desirable. Chromosome numbers of *V. scutellata* have been investigated from 27 localities throughout its entire distribution range in the northern parts of the Northern Hemisphere. All counts demonstrate that it is a diploid species ($2n = 2x = 18$).

The third section in subgenus *Veronica*, *Veronica* sect. *Montanae* (Boriss. ex Elenevsky) Assejeva, includes a group of Chinese–Himalayan species and *V. montana*, with the latter as sister to the rest (Albach, unpublished data). *Veronica montana* is diploid ($2n = 2x = 18$; 10 counts) almost throughout its distribution in Europe, with only a single tetraploid ($2n = 4x = 36$) found in France (Seidenbinder & Verlaque in Löve, 1985). The Chinese–Himalayan group in section *Montanae* is very poorly investigated. Only four of more than 20 species of subsection *Canae* (T. Yamaz.) Elenevsky have been investigated for chromosome numbers, and no clear pattern is present. A base chromosome number reduction to $x = 8$ seems to have occurred in *V. henryi* T. Yamaz. (tetraploid, $2n = 4x = 32$) and *V. miqueliana* Nakai (hexaploid, $2n = 6x = 48$) based on single reports for each. Two reports are available for *V. cana* Wall. with $2n = 50$ and $2n = 52$, which may be cases of increased chromosome number from $x = 8$, reductions from $x = 9$, or miscounts. Unfortunately, neither count is accompanied by a figure, which could have resolved the conflict. The chromosome number of the fourth species that has been investigated, $2n = 42$ for *V. robusta* (Prain) T. Yamaz., is also difficult to explain, but again may represent a miscount or an aberrant aneuploid individual, much less likely an independent reduction to $x = 7$. The scarcity and ambiguity of the data indicate the need to reinvestigate chromosome numbers coupled with DNA sequence data in order to infer the evolutionary trends in this group.

Veronica sect. *Veronica* can be divided in *Veronica* subsect. *Alpinae* Benth. (8 species), *V.* subsect. *Veronica* (5 species), and five other species across four other subsections. All these species have chromosome numbers based on $x = 9$ with the exception of *V. baumgartenii* Roem. & Schult. ($x = 7$; see below). Within *Veronica* subsect. *Alpinae*, *V. alpina* L., *V. nutans* Bong., *V. copelandii* Eastw., *V. stelleri* Pall. ex Link, and *V. nipponica* Makino are diploids ($2n = 2x = 18$; 37, nine, one, three, and one count, respectively, with one intrapopulational cytotype mixture: $2x$ and $4x$ in *V. alpina* in Norway; Knaben & Engelskjon, 1967), whereas *V. wormskjoldii* Roem. & Schult. is exclusively tetraploid ($2n = 4x = 36$; four counts). *Veronica bellidioides* is mostly tetraploid ($2n = 4x = 36$; 36 counts including ours from the Pyrenees and Bulgaria), but diploid plants ($2n = 2x = 18$; seven counts) are known from the

western Pyrenees (Küpfer, 1968, 1974). Sampling is adequate in most species of subsection *Alpinae* except for *V. stelleri*, which has not been sampled in Alaska, U.S.A., and *V. nutans*, which has only been studied in California and Nevada, U.S.A., as well as in Canada but not in the northern Cascades or the phylogeographically important (Albach et al., 2006) southern Rocky Mountains. *Veronica cusickii* A. Gray is more problematic, with only two populations from the distributional extremes studied, one diploid ($2n = 2x = 18$) from California and one octoploid ($2n = 8x = 72$) from southwestern British Columbia.

Veronica officinalis L. has been studied intensively throughout Europe but not beyond the Siberian part of its range. Most chromosome numbers reported are tetraploid ($2n = 4x = 36$; more than 80 counts), with diploids ($2n = 2x = 18$) found in Portugal (Muñoz-Centeno et al., 2007), Gotland/Sweden (Böcher, 1944), and in the east Siberian Sayan Mountains (Stepanov, 1994), the only count outside Europe. Reports of $2n = 32$ or 34 (Gadella & Kliphuis, 1963, 1966; Kliphuis & Wieffering, 1972; Rossitto et al., 1983) likely represent miscounts (voucher specimens for the first three studies have been checked; Albach, unpublished data). However, this is difficult to prove since only Rossitto et al. (1983) presented a figure, which arguably shows 35 chromosomes. The other four species in subsection *Veronica* have a fairly restricted distribution. *Veronica allionii* Vill. from the southwestern Alps is diploid ($2n = 2x = 18$; three counts), whereas *V. onoei* Franch. & Sav. from Japan is tetraploid ($2n = 4x = 36$; two counts). *Veronica dabneyi* Hochst. from the Azores and *V. morrisonicola* Hayata from Taiwan have not been studied yet.

The five remaining species in subgenus *Veronica* are more problematic. *Veronica aphylla* L. has been reported as diploid ($2n = 2x = 18$; 11 counts) in the Alps, Pyrenees, and Tatra mountains. The closely related *V. grandiflora* Gaertn. from around the Bering Strait has been reported to have $2n = 48$ 50 chromosomes, a number that cannot be associated with a regular ploidy level based on $x = 9$. *Veronica urticifolia* Jacq. is diploid ($2n = 2x = 18$; eight counts). Reports of $2n = 64$ (Meškova, 1965; Nilsson & Lassen, 1971) probably refer to misidentified *V. teucrium* L., although at least the voucher of one study (Nilsson & Lassen, 1971) was confirmed to be *V. urticifolia* (Fischer, unpublished data). A count of $2n = 16$ by Mattick (in Tischler, 1950) appears dubious and impossible to verify since no voucher specimen is indicated. Three studies have published chromosome numbers for *V. ponae* Gouan from the Pyrenees and northern Spain. It was reported as hexaploid ($2n = 6x = 54$; Küpfer, 1972, 1974) in five populations (also confirmed by us, see Table 1) and diploid ($2n = 2x =$ 16–18; Huber, 1927) in one. Unfortunately, no voucher specimen for the diploid count, which was grown in a botanical garden (Dilger-Endrulat, at TUB, pers. comm.), is known. It may be a misidentified *V. urticifolia*. Finally, *V. baumgartenii* (from the Carpathians) is inferred to have the deviating chromosome base number $x = 7$ ($2n = 2x = 14$; two counts), although it is mostly considered closely related to *V. aphylla*, a relationship not seen in DNA sequence analyses (Albach, unpublished data).

II. *VERONICA* SUBG. *BECCABUNGA*

This subgenus consists of three sections. The aquatic species of section *Beccabunga* (Hill) Dumort. are sister to sections *Acinifolia* (Römpp) Albach and *Serpyllifolia* G. Don (Albach et al., 2004a, 2005a; Fig. 2). Whereas species of the former section have a chromosome base number of $x = 9$, the plesiomorphic number for the subgenus (Albach et al., 2004a) and species of the other two sections have a base number of $x = 7$. Our results of $2n = 2x = 18$ and $2n = 2x = 36$ ($x = 9$) for *V. hispidula* Boiss. & Huet. are important in that respect because it has been considered to be a member of section *Acinifolia* (Römpp, 1928; Fischer, 1972). The different chromosome base number, however, agrees with its position in analyses of DNA sequences in which it is sister to the rest of the subgenus (Albach, unpublished data; see Fig. 2). It is therefore considered as having an uncertain position within the subgenus here.

Species boundaries in section *Beccabunga* have been interpreted differently by different authors (Borissova, 1955; Fischer, 1981); the species are treated here in a narrow sense with 13 species in the section. Vagueness of species boundaries is exemplified by—and based on—the apparent existence of different ploidy races in several species, which could either be due to cryptic taxa not recognized on basis of morphology or due to misidentification or misinterpretation of taxa. In subsection *Beccabunga*, *Veronica beccabunga* L. is diploid ($2n = 2x = 18$; subspecies *beccabunga* L., more than 50 counts; subspecies *abscondita* M. A. Fisch., three counts; subspecies *muscosa* (Korsh.) Elenevsky, one count), but tetraploid numbers ($2n = 4x = 36$) have been reported for subspecies *beccabunga* from Poland (Sokolowska in Skalinska, 1964), Italy (Ferrarella et al., 1981), and Sweden (Lökvist & Hultgard, 1999) and most likely represent autotetraploids. Counts of $2n = 2x = 16$ for subspecies *beccabunga* (Davlianidze, 1980) and subspecies *muscosa* (Sokolovskaja & Strelkova, 1939 according to Agapova et al., 1993) are probably miscounts. The close relative *V. americana* Schwein. ex Benth. is tetraploid ($2n = 4x = 36$; 12 counts).

Veronica anagallis-aquatica L. and its relatives are morphologically more difficult to differentiate, which is reflected in incorrectly determined specimens used for chromosome counts. *Veronica lysimachioides* Boiss. seems to be a diploid species ($2n = 2x = 18$; six counts), whereas *V. anagallis-aquatica*, *V. michauxii* Lam., and *V. catenata* Pennell are tetraploids ($2n = 4x = 36$; more than 70, five and 16 counts, respectively) and *V. undulata* Wall. is hexaploid ($2n = 6x = 54$; 12 counts). *Veronica oxycarpa* Boiss. seems to be another tetraploid species ($2n = 4x = 36$; six counts), but a diploid count ($2n = 2x = 18$) is reported by Meškova (1965; see Öztürk & Fischer [1982] for doubts regarding this count). The correct chromosome number for *V. poljensis* Murb. is unknown. Marchant (1970) states that it is diploid ($2n = 2x = 18$), but his figures show it to be tetraploid. Öztürk and Fischer (1982) report a tetraploid number, but state that their specimen is intermediate between *V. poljensis* and *V. anagalloides* Guss. Dzhus and Dmitrieva (2001) report a diploid from Belarus, but the species is not known from that area or near it and, therefore, may be a misidentified *V. anagalloides*. This species is diploid ($2n = 2x = 18$; subspecies *anagalloides* Guss., 10 counts; subspecies *heureka* M. A. Fisch., 10 counts including the report here). However, several tetraploid counts have been published. Meškova's (1965) tetraploid count ($2n = 4x = 36$) from Ukraine has been considered improbable by Öztürk and Fischer (1982), and no voucher could be located. The voucher specimen for the tetraploid count from Afghanistan (Podlech & Dieterle, 1969) does not resemble the typical subspecies *heureka* (Öztürk & Fischer, 1982), but the one from Yemen (Podlech, 1986) does (Albach, pers. obs.). The voucher specimens from Romania (Vasudevan, 1975), Pakistan (Khatoon, 1991, in Khatoon & Ali, 1993), and Iran (Saeidi-Mehrvarz & Kharabian, 2005) have not been checked. Information on the voucher is only given for the number from Iran. Based on this information, it is probable that tetraploid taxa of *V. anagalloides* subsp. *heureka* ($2n = 4x = 36$) exist in southwest Asia. Sánchez-Agudo, Delgado, and Martínez-Ortega (in prep.) recently found tetraploid chromosome numbers ($2n = 4x = 36$) from Spain. Their status needs further attention. The same is true for tetraploid counts of *V. anagalloides* subsp. *heureka* from Russian Far East ($2n = 4x = 36$; Probatova et al., 1996), which is by far the easternmost occurrence of this taxon. Differentiation of this taxon is often difficult as plants often resemble diminutive *V. anagallis-aquatica*. *Veronica scardica* Griseb. is another species for which two ploidy levels are reported. Marchant (1970) reports diploid chromosome numbers ($2n = 2x = 18$) from several countries without further documentation. This ploidy level was also reported by Öztürk and Fischer (1982) from Turkey. Strid and Franzén (in Löve, 1981) and van Loon and van Setten (in Löve, 1982), however, report tetraploid plants ($2n = 4x = 36$) from the Balkan Peninsula. The identity of the voucher specimen for the first tetraploid plant has been confirmed; the one for the second shows a mixture of characters and is not clearly identifiable but is definitely not pure *V. scardica*. *Veronica scardica* is a serpentinophyte that might be only an ecological race of *V. anagalloides* or *V. anagallis-aquatica* (Fischer et al., 1984), which could explain the different ploidy levels reported. Detailed morphological and karyological analyses together with cultivation experiments will be necessary to solve the systematic questions regarding this species.

Finally, the annual *Veronica peregrina* L., the only member of subsection *Peregrinae* Elenevsky, was also shown to be a member of section *Beccabunga* (Albach & Chase, 2001; Albach et al., 2004a). Eleven counts confirm its chromosome number of $2n = 6x = 52$. which was hypothesized to be derived from $2n = 6x = 54$ by chromosome fusion (cf. Hofelich, 1935; Albach et al., 2004a). The count of $2n = 54$ by Chuang and Heckard (1992) from Oregon is interesting in that respect because it may represent a lineage that diverged before the fusion of the chromosomes occurred. Alternatively, it may be a miscount, but no figure is included in the publication.

Section *Acinifolia* comprises eight species (without *Veronica hispidula*, which we exclude). Previously, chromosome numbers were known for four species, the diploid ($2n = 2x = 14$) *V. acinifolia* L., *V. pusilla* Kotschy & Boiss., and *V. syriaca* Roem. & Schult. (11, three and three counts, respectively) and the tetraploid *V. bozakmanii* M. A. Fisch. ($2n = 4x = 28$; six counts including the report here). We add here information on a fifth species, *V. reuteriana* Boiss., for which within one population two plants showed the tetraploid level ($2n = 4x = 28$) and one plant the hexaploid level ($2n = 6x = 42$). Intraspecific ploidy level variation in *V. hispidula* and *V. reuteriana* is noteworthy. No morphological character corresponding to different ploidy levels has been noted but should be looked for in future investigations. Chromosome numbers in other rare species from southwest Asia are still unknown.

Sister to section *Acinifolia* is section *Serpyllifolia* in subgenus *Beccabunga*, a group including only perennial taxa, which probably shares the ancestral chromosome base number of $x = 7$ (Fig. 2). This subsection includes 11 species. Chromosome numbers are available for five taxa. These include the diploid *V. telephiifolia* Vahl ($2n = 2x = 14$; one count), *V.*

serpyllifolia L. (more than 70 counts; including subspecies *repens* (Clarion ex DC.) Hartl in Hegi from Corsica), and *V. nevadensis* var. *langei* (Lacaita) M. M. Mart. Ort. & E. Rico (six counts), which form subsection *Serpyllifolia* (G. Don) Stroh. Mattick (in Tischler, 1950) and Peev (1975) report tetraploid counts ($2n = 4x = 28$) for *V. serpyllifolia* from Austria and Bulgaria, respectively, which may be spontaneous autotetraploids, but a voucher specimen for the first report is not indicated and those from Peev have not been checked. A count of $2n = 2x = 16$ by Probatova and Sokolovskaya (1990) is probably a miscount. Unfortunately, no figure is presented.

The polyploid complex of *Veronica gentianoides* Vahl, classified as subsection *Gentianoides* (G. Don) Assejeva, is without a doubt the most complex group karyologically in the genus. Eight different ploidy levels on two different base numbers have been published, and our report of $2n = 36$ presents the ninth ploidy level (with a range of $2x$–$10x$). Tumadjanov has studied this group extensively (e.g., Tumadjanov & Beridze, 1969; Tumadjanov et al., 1977), but questions still remain, partly due to the limited documentation of chromosome counts in his studies. Tumadjanov et al. (1977) proposed an intraspecific base number switch from $x = 8$ to $x = 12$, with $x = 8$ predominating in the Turkish and Armenian distribution area and $x = 12$ in the Greater Caucasus and Georgian Lesser Caucasus. However, this distinction is refuted by his own data. The ancestral diploid race ($2n = 2x = 16$) is found in the northern Colchis, a suggested refugium for tertiary forest species (Tumadjanov & Beridze, 1969). Other chromosome numbers based on $x = 8$ found in the Greater Caucasus are: $2n = 4x = 32$ (throughout the species range; Tumadjanov & Beridze, 1969; Tumadjanov et al., 1972), $2n = 5x = 40$ (Tumadjanov et al., 1972), $2n = 7x = 56$ (Western Caucasus; Magulaev, 1984), $2n = 8x = 64$ (Azerbaijan; Tumadjanov & Beridze, 1969 from Central Caucasus; Kliphuis in Löve, 1979), and $2n = 10x = 80$ (Georgian parts of the Lesser Caucasus; Tumadjanov & Beridze, 1969; Tumadjanov et al., 1972). Other chromosome numbers of the Greater Caucasus are more compatible with a base number of $x = 12$. The related *V. schistosa* E. Busch ($2n = 24$; six counts) is either a diploid ($x = 12$) or a triploid ($x = 8$). Zakharjeva (in Agapova et al., 1993) counted $2n = 48$ (tetraploid or hexaploid, respectively) for this species, but no voucher specimen is indicated. Therefore, we cannot be sure about its identity, and it may be a count for the closely related *V. gentianoides* in which this number is common. According to Tumadjanov et al. (1972, 1977), the predominating polyploid races in *V. gentianoides* in the Greater Caucasus have $2n = 24$ (either $2x$ or $3x$), $2n = 48$ (either $4x$ or $6x$), and $2n = 72$ ($6x$ or $9x$), the latter two confirmed by us. Our report of $2n = 36$ is, however, the first that is incompatible with a hypothesis of a basic chromosome number of $x = 8$ in *V. gentianoides* in the Greater Caucasus and suggests instead $x = 12$. The predominating polyploid races in the Lesser Caucasus (especially Armenia) have $2n = 32$ and 48, although data from Turkey are lacking. Various species have been segregated from *V. gentianoides* by some authors (e.g., Kemularia-Nathadze, 1955), but no information on ploidy level for these segregates is available.

III. *VERONICA* SUBG. *PSEUDOLYSIMACHIUM*

This subgenus includes 26 species, with chromosome numbers known for 18. In contrast to the case in *Veronicastrum* and *Picrorhiza*, the derived chromosome base number $x = 17$ likely originates from the combination of two genomes with $2n = 18$ with a subsequent reduction to $2n = 17$ based on its phylogenetic position distant to any taxon with $x = 8$ (Fig. 2) and karyological observations by Graze (1935). The origin of the base chromosome number $x = 17$ has been further discussed by Albach et al. (2004a). To highlight the fact that it is a derived base chromosome number for *Veronica* subg. *Pseudolysimachium* alone, it will subsequently be denoted as x_{Psl} ($= 2x$). Seven of the species in the subgenus are diploid ($2n = 2x_{Psl} = 34$), two are tetraploid ($2n = 4x_{Psl} = 68$), and seven include both diploid and tetraploid populations. The tetraploid level for *V. spuria* L. (Peev in Löve, 1972b) needs confirmation because unfortunately voucher information given in the publication and specimens in the herbarium do not match (M. A. Fischer, pers. obs.). Chromosome numbers of the European species have recently been reviewed by Travníček et al. (2004) and Albach and Fischer (2003). In Asia, only diploids ($2n = 2\ x_{Psl} = 34$) of *V. daurica* Steven, *V. linariifolia* Pall. ex Link, *V. kiusiana* subsp. *miyabei* (Nakai & Hondo) T. Yamaz., *V. nakaiana* Ohwi, *V. pinnata* L., and *V. schmidtiana* Regel and tetraploids ($2n = 4\ x_{Psl} = 68$) of *V. kiusiana* subsp. *maritima* (Nakai) T. Yamaz., *V. ornata* Monjuschko, and *V. subsessilis* (Miq.) Carriere are known mostly from single counts. Chromosomes of the species in this subgenus are extremely small and sticky, which makes the correct determination of chromosome numbers difficult (cf. Weiss et al., 2002). Deviations from $2n = 2x_{Psl} = 34$ or $2n = 4\ x_{Psl} = 68$ by one or two chromosomes are consequently more common than in other subgenera. Unfortunately, the first publication of chromosome numbers in *Veronica* (Heitz, 1926) already includes a dubious count, $2n = 48$ for *V. azurea* Link (a synonym of *V. longifolia* L.).

The figure shows 48 to 50 chromosomes, but clearly not the expected 34 or 68 chromosomes. No voucher information is included, so this count remains doubtful. Other doubtful records that require further investigation are those for *V. grandis* Fisch. ($2n = 56$; Zhukova, 1967, according to Agapova et al., 1993), *V. olgensis* Kom. ($2n = 24$; Gurzenkov, 1973, likely to be a printing error for $2n = 34$), and *V. sajanensis* Printz ($2n = 18$; Malachova, 1971; Krasnoborov, 1976; both according to Krogulevich and Rostovtseva, 1984) and those by Androshchuk (1988). The counts by Androshchuk (1988) appear to have a systematic error because nine different species have been investigated and all counts are based on $x = 9$ and not on $x = 17$ as expected for subgenus *Pseudolysimachium*. A count of $2n =$ ca. 90 for *V. longifolia* from Yakutia by Krogulevich and Rostovtseva (1984) also appears dubious. Future research on the European species of this subgenus needs to focus on the origin of tetraploids, whereas more fundamental information on chromosome numbers and morphology is necessary for the Asian species of the subgenus.

IV. *VERONICA* SUBG. *SYNTHYRIS*

Chromosome numbers are known for all species of *Veronica* subg. *Synthyris*. They all share the chromosome base number of $x = 12$, which is rare in Veroniceae with all extant relatives suggested by phylogenetic analyses (e.g., Albach et al., 2004a; Fig. 2) having $x = 9$. Lepper (1964), based on karyotype analysis, suggested that the number represents a diploid level with aneuploid chromosome number increase. However, the alternative that the number is derived from an ancestor with $2n = 2x = 14$ followed by reduction to $n = 6$ in gametes and perhaps production of unreduced gametes and subsequent polyploidization should not be discarded because of the frequent parallel reductions to $x = 7$ in *Veronica* (see above). Most species are diploid ($2n = 2x = 24$), but *V. ritteriana* (Eastw.) M. M. Mart. Ort. & Albach and *V. canbyi* (Pennell) M. M. Mart. Ort. & Albach are tetraploid ($2n = 4x = 48$), and, in *V. missurica* subsp. *major* (Hook.) M. M. Mart. Ort. & Albach, *V. missurica* subsp. *stellata* (Pennell) M. M. Mart. Ort. & Albach, *V. plantaginea* E. James, and *V. wyomingensis* (A. Nelson) M. M. Mart. Ort. & Albach, both diploid and tetraploid plants have been found in different populations.

V. *VERONICA* SUBG. *COCHLIDIOSPERMA*

Veronica subg. *Cochlidiosperma* is the group with the most counts per species. With the exception of *V. sibthorpioides* Deb., Degen & Hervier, all species share the chromosome base number $x = 9$. *Veronica sibthorpioides*, a species from southern Spain and Morocco, however, has $2n = 30$ as confirmed by 14 counts from different localities, although Sánchez-Agudo et al. (unpublished data) recently reported two counts of $2n = 28$. Several species are diploid ($2n = 2x = 18$): *V. crista-galli* Steven (three counts), *V. stewartii* Pennell (two counts), *V. triloba* Opiz (25 counts), *V. stamatiadae* M. A. Fisch. & Greuter (one count), *V. lycica* E. B. J. Lehm. (one count), *V. panormitana* Tineo (six counts), and *V. trichadena* Jord. & Fourr. (seven counts). A count (Peev in Löve, 1972a) from Bulgaria of *V. hederoides* M. A. Fisch. (a synonym of *V. stewartii* from the Himalayas; $2n = 2x = 18$) refers to *V. triloba* (Fischer, 1984). Counts of $2n = 2x = 18$ for *V. sublobata* M. A. Fisch. and *V. hederifolia* L. by Nordenstam and Nilsson (1969) and Guo and Liu (2001), respectively, probably also refer to *V. triloba*, but the vouchers have not been checked. The proposed separation of *V. sublobata* from *V. hederifolia* s. str. (Fischer, 1967), partly based on its lower ploidy level (tetraploid vs. hexaploid), has led to numerous new reports (more than 100 populations counted for each taxon) for these two species, especially in The Netherlands (de Jongh & Kern, 1973; Gadella & Kliphuis, 1976) and northern Europe (Nordenstam & Nilsson, 1969; Fischer, 1975b), showing a rather perfect correlation between tetraploidy in *V. sublobata* and hexaploidy in *V. hederifolia* s. str. and a clear-cut separation between these species. The few early deviating counts in northern Europe (Nordenstam & Nilsson, 1969) were due to insufficiently careful identification of the investigated specimens. Morphological differentiation between *V. sublobata* and *V. hederifolia* s. str. seems to be more difficult, however, in some southern regions of Europe where chromosome counts are still scarce (Sánchez-Agudo et al., unpublished data). *Veronica sublobata* is a tetraploid ($2n = 4x = 36$) derivative of *V. triloba* (Fischer, 1975b; Albach, unpublished data). Hybridization between *V. triloba* and *V. sublobata* gave rise to the hexaploid ($2n = 6x = 54$) *V. hederifolia* (Albach, unpublished data). A count of $2n = 56$ by Meškova (1965) is probably a miscount, but unfortunately, this publication was inaccessible to us. Counts of $2n = 26$ to 28 (Sorsa, 1963; Gadella & Kliphuis, 1966) probably refer to *V. persica*, which often grows side by side with *V. hederifolia*. No voucher is indicated by Sorsa (1963) and the voucher from Gadella and Kliphuis (1966) has not been checked. A tetraploid count ($2n = 4x = 36$) for *V. hederifolia* from Iran (Saeidi-Mehrvarz & Kharabian, 2005) is of interest because *V. sublobata* is not known from that region. This may represent a different taxon and requires further confirmation. A publication from Guo and Liu

(2001) provided a range of chromosome numbers for *V. hederifolia* from its introduced range in Nanjing, China, including diploids ($2n = 2x = 18$), tetraploids ($2n = 4x = 36$), and hexaploids ($2n = 6x = 54$), as well as more unexpected numbers: $2n = 22, 32$. Their drawings in the paper indicate the reported chromosome numbers. It is not clear how an aneuploid chromosome race could have arisen in the short time since its introduction, and a mix-up of the samples with another weedy species with $2n = 32$ during the collection or processing is considered most likely.

Veronica cymbalaria Bodard is karyologically more difficult. Tetraploid ($2n = 4x = 36$) and hexaploid ($2n = 6x = 54$) plants are morphologically indistinguishable (Fischer, 1975a), and both ploidy levels originated more than once from *V. panormitana* and two different clades of *V. trichadena* (Albach, 2007). A map of the origins of 38 known tetraploid and 24 known hexaploid plants of *V. cymbalaria* will be published elsewhere. Counts of $2n = 2x = 18$ by Hofelich (1935) and Nilsson and Lassen (1971) probably refer to *V. trichadena* or *V. panormitana*, but a voucher for the first could not be found (Dilger-Endrulat, at TUB, pers. comm.; Albach, pers. obs.) and the other has not been checked.

VI. *VERONICA* SUBG. *PELLIDOSPERMA*

This subgenus is a small group of seven annual species. Chromosome numbers for *Veronica donii* Römpp, *V. aznavourii* Dörfl., and *V. samuelssonii* Rech. f. are not available but would be highly desirable because, amazingly, three different base numbers ($x = 7, 8, 9$) have been reported so far in this subgenus with all taxa reported as diploid. *Veronica triphyllos* L. has $2n = 2x = 14$ (18 counts); *V. praecox* All. and *V. glauca* Sibth. & Sm. have $2n = 2x = 18$ (19 and five counts, respectively); and *V. mazanderanae* Wendelbo, a species endemic to Iran, has $2n = 2x = 16$ (one count).

VII. *VERONICA* SUBG. *STENOCARPON*

This subgenus includes 31 montane to alpine species of Eurasia and Mexico. Chromosome numbers are known for all eight European species but only for two Asian species (*V. ciliata* Fisch., *V. densiflora*). All chromosome numbers are based on $x = 8$ and represent diploids ($2n = 2x = 16$) with the exception of the tetraploid ($2n = 4x = 32$) *V. contandriopouli* Quézel from Greece, which is possibly just an aberrant specimen of autotetraploid *V. erinoides* Boiss. & Spruner. *Veronica fruticans* Jacq. is the best studied species (31 counts throughout its distribution area in the European high mountain ranges). For all other species, less than five counts per species have been published (*V. ciliata*, four counts including the report here; *V. densiflora*, four counts; *V. erinoides*, one count; *V. fruticulosa* L., three counts; *V. mampodrensis* Losa & P. Monts., one count; *V. nummularia* Gouan, three counts including the report here; *V. saturejoides* Vis., three counts; *V. thessalica* Benth., two counts). Our count of $2n = 2x = 16$ for *V. ciliata* from the southeasternmost distribution range confirms those from other extremes of the distribution.

VIII. *VERONICA* SUBG. *POCILLA*

Veronica subg. *Pocilla* (Dumort.) M. M. Mart. Ort., Albach & M. A. Fisch. includes 27 annual and one perennial species including the well-known cosmopolitan weed *V. persica*. The chromosome base number for the 15 species counted is $x = 7$, although it is not clear for *V. cardiocarpa* (Kar. & Kir.) Walp., for which the only publication states $2n = 14–16$ (Hofelich, 1935), and *V. campylopoda* Boiss. (see below). Chromosome numbers are known for over half of the species, especially the weedy species (*V. persica*, 48 counts; *V. polita* Fr., 38 counts; *V. agrestis* L., 22 counts; *V. filiformis* Sm., 12 counts; and *V. opaca* Fr., 11 counts). Diploids ($2n = 2x = 14$) include *V. polita* (sometimes under its synonym *V. didyma* Ten.), *V. cardiocarpa* (one count), *V. ceratocarpa* C. A. Mey. (three counts), *V. filiformis*, *V. francispetae* M. A. Fisch. (two counts), and *V. siaretensis* E. B. J. Lehm. (one count). *Veronica persica*, *V. agrestis*, *V. opaca*, *V. biloba* L. (three counts), *V. capillipes* Nevski (two counts), and *V. rubrifolia* Boiss. (two counts) are tetraploids ($2n = 4x = 28$). Taxonomic history, especially in Asia, confused *V. agrestis* with *V. polita* for a long time (Lehmann, 1910, 1940), and, consequently, several publications from the same authors in India state a diploid level for *V. agrestis* (e.g., Bir & Sidhu in Löve, 1978). Probably all of these publications refer to *V. polita*, which is common in Asia, rather than *V. agrestis*, which occurs exclusively in Europe. A report of $2n = 2x = 18$ for *V. polita* from Hungary (Borhidi, 1968) is probably due to confusion with *V. triloba*, but no voucher specimen is indicated.

Regarding *Veronica* sect. *Subracemosae* (Benth.) Assejeva in subgenus *Pocilla*, our count of $2n = 6x = 42$ is the third report confirming the hexaploid level for *V. arguteserrata* Regel & Schmalh. *Veronica campylopoda* is the only species in this subgenus and one of four in the genus for which more than two ploidy levels have been reported in the literature. Fischer (1981) reported a tetraploid ($2n = 4x = 28$) plant from Iran. Nine counts of hexaploid plants ($2n = 6x = 42$) originate from Central Asia to the Mediterranean, with our count being the first from Turkey. Our report of an

octoploid plant ($2n = 8x = 56$) is the second for that ploidy level in *V. campylopoda*, with both notably from Turkey. Reports of $2n = 2x = 18$ from Afghanistan (Podlech & Dieterle, 1969) and $2n = 4x =$ ca. 36 from Utah, U.S.A. (Bell, 1965), where it is introduced, appear dubious in light of other reports, but voucher specimens for the first count have been confirmed by the first author. The voucher specimen for the second belongs to *V. arguteserrata*. However, such morphological intergradation is typical for well-watered specimens of *V. campylopoda* (Albach, unpublished data).

IX. *VERONICA* SUBG. *PENTASEPALAE*

Subgenus *Pentasepalae* (Benth.) M. M. Mart. Ort., Albach & M. A. Fisch. is by far the largest subgenus of *Veronica* in the Northern Hemisphere, with 72 species most common in southwest Asia. Chromosome numbers are available for 32 taxa including all European species. All species investigated have a base chromosome number of $x = 8$, including 19 diploids ($2n = 2x = 16$), five tetraploids ($2n = 4x = 32$), one hexaploid ($2n = 6x = 48$), and three octoploids ($2n = 8x = 64$). European and Siberian species are classified within *Veronica* subsect. *Pentasepalae* Benth. and represent several, often ancestral, diploid species (*V. crinita* Kit. ex Schult., two counts; *V. kindlii* Adamovíc, one count; *V. krylovii* Schischk., one count; *V. orbelica* (D. Peev) D. Peev, one count; *V. orsiniana* Ten., 14 counts; *V. prostrata* L., 20 counts including the reports here; *V. rhodopea* (Velen.) Degen ex Stoj. & Stef., one count; *V. rosea* Desf., 13 counts; *V. tenuifolia* Asso, 16 counts; *V. turrilliana* Stoj. & Stef., three counts). Polyploid species include *V. aragonensis* Stroh, *V. scheereri* (J. P. Brandt) Holub ($2n = 4x = 32$; six and 24 counts, respectively), and *V. sennenii* (Pau) M. M. Mart. Ort. & E. Rico ($2n = 8x = 64$; six counts). For the three most widespread species of *Veronica* subsect. *Pentasepalae*, several ploidy levels have been reported. *Veronica teucrium* is octoploid ($2n = 8x = 64$) with one publication reporting diploid plants ($2n = 2x = 16$) from southern France (Küpfer, 1969), possibly belonging to *V. orsiniana*, and one hexaploid plant ($2n = 6x = 48$) from Germany (Lippert & Heubl, 1989). Voucher specimens for the hexaploid plants have been checked and represent *V. teucrium* rather than the sympatric *V. austriaca* L., which is mostly hexaploid except for some specimens from southwestern Germany and northwestern Switzerland (Brandt, 1952, 1961). However, intermediates between *V. teucrium* and *V. austriaca* are known from southwestern Germany; therefore, these populations need further study in a wider context. The third species of the subsection, which is widespread and karyologically polymorphic, is *V. jacquinii* Baumg., for which octoploid plants ($2n = 8x = 64$) are reported from Greece (Strid in Löve, 1986b). Other reports for this species are mostly hexaploid ($2n = 6x = 48$; 13 counts), with a diploid race ($2n = 2x = 16$) reported from Albania (Baltisberger, 1988) and tetraploid ($2n = 4x = 32$) and decaploid races ($2n = 10x = 80$) reported from Bulgaria (Peev, 1972). Pinnatifid leaves have evolved multiple times independently in *Veronica* (Albach et al., 2004c) and may even have evolved independently in taxa of different ploidy levels, but all are considered here under *V. jacquinii*.

The same is true for *Veronica multifida* L. of *Veronica* subsect. *Orientales* (Wulff) Stroh, a taxon with species mainly found in Turkey. Diploid plants ($2n = 2x = 16$) of *V. multifida* have been reported from Bulgaria (Peev in Löve, 1972b) and southern Turkey (Fischer, 1970), tetraploids ($2n = 4x = 32$) from Armenia (Meškova, 1965) and southern Turkey (Fischer, 1970), hexaploids ($2n = 6x = 48$) from southwestern Turkey (Fischer, 1970), and decaploids ($2n = 10x = 80$) from Armenia (Gukasian & Safarian, 1990). *Veronica multifida* is a highly polymorphic species, but no morphological correlate to the different ploidy races has been found (Fischer, 1970). In this respect, *V. multifida* resembles *V. orientalis* Mill., another morphologically polymorphic species from southwest Asia with several ploidy levels reported. A tetraploid plant ($2n = 4x = 32$) has been reported from Armenia (Meškova, 1965) and octoploids ($2n = 8x = 64$) from Iran (Ghaffari in Löve, 1986a) and Israel (Pazy, 2000). The closely related *V. kurdica* Benth. is hexaploid ($2n = 6x = 48$; one count). Other species of the subsection are mostly diploid ($2n = 2x = 16$; *V. bombycina* Boiss. & Kotschy, two counts including the report here; *V. caespitosa* Boiss., two counts; *V. cinerea* Boiss. & Balansa, two counts; *V. cuneifolia* D. Don, one count; *V. dichrus* Schott & Kotschy, three counts; *V. farinosa* Hausskn., one count; *V. macrostachya* Vahl subsp. *sorgerae* M. A. Fisch., one count; *V. pectinata* L., one count) with only *V. elmaliensis* M. A. Fisch. being octoploid ($2n = 8x = 64$, one count).

Chromosome numbers for the ca. 30 species of the five remaining subsections of *Veronica* subg. *Pentasepalae* are lacking except for two, the diploid taxon *V. peduncularis* M. Bieb. ($2n = 2x = 16$, three counts) and the tetraploid *V. microcarpa* Boiss. ($2n = 4x = 32$, one count). Future research should especially focus on southwest Asia and the Caucasus since those taxa for which no chromosome number is yet available occur in Turkey (18 species), Iran (12 species), and the Greater Caucasus (10 species).

X. *VERONICA* SUBG. *CHAMAEDRYS*

Veronica subg. *Chamaedrys* is one of the best studied groups in *Veronica* including one of the best studied species (*V. chamaedrys* L., with more than 90

counts). All species have chromosome numbers based on $x = 8$ except for *V. magna*, which appears to have $x = 7$ (see below). Only *V. sartoriana* Boiss. & Heldr., a Greek endemic closely related to *V. arvensis* L., has not been studied. The annual members of this subgenus, *V. arvensis* (42 counts), *V. verna* L. (17 counts), *V. dillenii* Crantz (12 counts), and *V. brevistyla* Moris (two counts), are all diploid ($2n = 2x = 16$). A count of $2n = 14$ for *V. arvensis* was reported by Löve and Löve (1956) from Iceland. Unfortunately, neither a figure of the chromosomes is given nor a voucher specimen indicated. Therefore, we cannot check our assumption of a miscount or a mix-up with *V. polita*. The European perennial species are also diploid with the exception of *V. chamaedrys* subsp. *chamaedrys*, which is tetraploid ($2n = 4x = 32$). However, within *V. chamaedrys* subsp. *chamaedrys*, some diploid taxa are insufficiently known (Fischer, 1973; see Dobeš & Vitek, 2000 for a map) and several diploid plants cannot be clearly assigned to the described taxa (Bardy & Albach, unpublished data). Finally, the Caucasian *V. magna* seems to be hexaploid ($2n = 6x = 42$) and the Asian *V. laxa* Benth. has been reported to be either tetraploid ($2n = 4x = 32$; China) or hexaploid ($2n = 6x (-2) = 46$; Japan, India). More information on the latter would be desirable to investigate whether tetraploids and hexaploids can be differentiated and whether diploid progenitor taxa still exist within the species.

XI. *VERONICA* SUBG. *PSEUDOVERONICA* (HEBE COMPLEX)

Veronica subg. *Pseudoveronica* J. B. Armstr., established by Armstrong (1881), is the nomenclaturally correct name for the Australasian species of *Veronica* formerly grouped in *Hebe* Comm. ex Juss. and related genera (Garnock-Jones et al., 2007). Chromosome numbers for species of *Parahebe* W. R. B. Oliv. on New Zealand (Garnock-Jones & Lloyd, 2004), *Hebe* (Bayly & Kellow, 2006), and the Australian species (Briggs & Ehrendorfer, 2006) have recently been reviewed. Therefore, we will only comment on the numbers of New Guinean species and the more general picture. The Australasian species constitute a monophyletic group nested within the $x = 8$ clade (Fig. 2) and apparently derived from a single polyploidization event. Thus, the derived chromosome base number in the subgenus is denoted as x_{Hebe} (= $6x$, see below). *Veronica* (= *Detzneria*) *tubata* (Diels) Albach from New Guinea, which is sister to the remaining Australasian species in a cladistic analysis of morphological characters (Hong, 1984) and in some of the DNA-based phylogenetic analyses by Albach et al. (2005a), has $2n = 48$ (Borgmann, 1964). This would fit in with a hexaploid origin of the Australasian species or $2n = 38$–42 (Briggs & Ehrendorfer, 2006), which is more in line with reports for other species in the subgenus with chromosome numbers derived from $x_{Hebe} = 20$ and $x_{Hebe} = 21$. Wagstaff and Garnock-Jones (1998) have shown that $x_{Hebe} = 21$ is ancestral to $x_{Hebe} = 20$, which evolved twice, once in the main clade of *Hebe* on New Zealand and once in Australia. Further phylogenetic analyses (Wagstaff et al., 2002; Albach et al., 2005a) have demonstrated that two further changes to $x_{Hebe} = 20$ need to be assumed for *V. decora* (Ashwin) Garn.-Jones in New Zealand and *V. ionantha* Albach in New Guinea. A hexaploid origin of the subgenus would therefore require an inferred loss of six to 12 chromosomes in the various species of the subgenus. Our understanding of phylogenetic relationships of Veroniceae in Australia, which shows much greater karyological diversity than the species in New Zealand, is unfortunately too limited to allow further inferences. The phylogenetic relationships of those taxa for which DNA sequences are available, inferred by Wagstaff et al. (2002), and their chromosome numbers are shown in Figure 2. The 12 species of *Parahebe* in New Guinea likely originate from a single immigration of a species from New Zealand (Albach et al., 2005a), chromosome numbers are available from a single publication (Borgmann, 1964) for two species (*V. ionantha* (= *P. ciliata* (Pennell) P. Royen & Ehrend.): $2n = 2x_{Hebe} = 40$; *V. albiflora*: $2n = 2x_{Hebe} - 42$).

XII. *VERONICA* SUBG. *TRIANGULICAPSULA*

This taxon includes two morphologically enigmatic annuals. They are difficult to place using molecular data (Albach et al., 2004a). Even karyologically, no relationship is discernible because both species, the diploid *Veronica grisebachii* Walters from Turkey and the tetraploid *V. chamaepithyoides* Lam. from Spain, have the unusual chromosome base number of $x = 6$.

THE REMAINING SPECIES IN *VERONICA*

Chromosome numbers are known for one species that has not been assigned to any subgenus. *Veronica javanica* Blume is $2n = 16$, similar to subgenus *Chamaedrys* with which it shares some morphological characters, especially seed ultrastructure (Muñoz-Centeno et al., 2006). However, neither nuclear ribosomal nor plastid DNA sequences show a relationship with the $x = 8$ clade (Albach et al., 2005a; Fig. 2), which suggests that it has gained this base number independently.

Chromosome numbers of the other species in *Veronica* that to date have not been placed confidently in any subgenus (*V. himalensis* D. Don, *V. monticola* Trautv., *V. ruprechtii* Lipsky, *V. simensis* Fresen., *V. tibetica* D. Y. Hong, and *V. viscosa* Boiss.) would be highly desirable.

NOMENCLATURAL CHANGES FOR *VERONICA*

For the purpose of providing a taxonomically ordered overview of chromosome numbers in Veroniceae, we provide a list (Appendix 1) resolved down to the subsectional level in *Veronica* including all species currently accepted by us. Three new names at the supraspecific level are used here for the first time, and the correct nomenclatural publication of these names is given here. The description of a new subsection for *V. anagallis-aquatica* and relatives will be published in the future.

Veronica sect. **Acinifolia** (Römpp) Albach, stat. nov. *Acinifolia* Römpp in Repert. Spec. Nov. Regni Veg. Beih. 50: 60. 1928 [*Veronica Verwandtschaftsgruppe*]. TYPE: *Veronica acinifolia* L., Sp. Pl., ed. 2, 1: 19. 1762.

The clade is equivalent at rank to *Veronica* sect. *Beccabunga* and *Veronica* sect. *Serpyllifolia* but has not been used at the sectional level yet.

Veronica sect. **Glandulosae** (Römpp) Albach, stat. nov. *Glandulosae* Römpp, in Repert. Spec. Nov. Regni Veg. Beih. 50: 33. 1928 [*Veronica Verwandtschaftsgruppe*]. TYPE: *Veronica glandulosa* Hochst. ex Benth., Prodr. (DC.) 10: 482. 1846.

The same rationale as above applies in this case.

Veronica subsect. **Cochlidiosperma** (Rchb.) Albach, stat. nov. *Cochlidiosperma* Rchb., Fl. Germ. Excurs.: 365. 1831–1832 [*Veronica* (unranked infragenus)]. TYPE (designated by Pouzar, 1964): *Veronica hederifolia* L., Sp. Pl. 1: 13. 1753.

Veronica hederifolia has traditionally been placed in subsections, whose type now belongs in subgenus *Pocilla*. To distinguish the blue-flowering species of *Veronica* sect. *Cochlidiosperma* from the white-flowering species (*Veronica* subsect. *Cymbalariae*), a new subsection needs to be established.

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Appendix 1. List of all species in the Veroniceae for which chromosome numbers are known. Chromosome numbers are expressed as $2n$ values and x refers to the ploidy level. Subscripts (x_{Vst}, x_{Pic}, x_{Psl}, x_{Hebe}) refer to the fact that these groups have a different chromosome base number because they are themselves derived from a polyploidization event. The list follows the classification of the tribe as outlined in Albach et al. (2004b) and Garnock-Jones et al. (2007).

Lagotis Gaertn.
$x = 11$
L. brevituba Maxim., $?x = 54$
L. cashmeriana (Royle) Rupr., $2x = 22$
L. glauca Gaertn., $2x = 22$
L. integrifolia (Willd.) Schischk., $4x = (43)\ 44$
L. minor (Willd.) Standl., $2x,4x$ 22, 44
L. stolonifera (K. Koch) Maxim., $2x = 22$
L. takedana Miyabe & Tatew., $2x = 22$

Species not investigated: *Lagotis alutacea* W. W. Sm., *L. angustibracteata* P. C. Tsoong & H. P. Yang, *L. brachystachya* Maxim., *L. chumbica* R. R. Mill, *L. clarkei* Hook. f., *L. crassifolia* Prain, *L. decumbens* Rupr., *L. globosa* (Kurz) Hook. f., *L. gregorjevi* Krassn., *L. hultenii* Polunin, *L. humilis* P. C. Tsoong & H. P. Yang, *L. ikonnokorii* Schischk., *L. integra* W. W. Sm., *L. kongboensis* T. Yamaz., *L. korolkowii* (Regel & Schmalh.) Maxim., *L. kunawurensis* (Royle ex Benth.) Rupr., *L. macrosiphon* P. C. Tsoong & H. P. Yang, *L. nepalensis* T. Yamaz., *L. pharica* Prain, *L. praecox* W. W. Sm., *L. ramalana* Batalin, *L. spectabilis* (Kurz) Hook. f., *L. uralensis* Schischk., *L. wardii* W. W. Sm., *L. yesoensis* Tatew., *L. yunnanensis* W. W. Sm.

Wulfenia Jacq.
$x = 9$
W. baldacii Degen, $2x = 18$
W. blecicii Lakušić, $2x = 18$
W. carinthiaca Jacq., $2x = 18$
W. orientalis Boiss., $2x = 18$

Paederota L.
$x = 9$
P. bonarota L., $4x = 36$
P. lutea Scop., $4x$, $6x = 36, 54$
P. ×churchillii Huter, $4x = 36$

Wulfeniopsis D. Y. Hong
$x = 8$
W. amherstiana (Benth.) D. Y. Hong, $2x$, $4x = 16, 32$
W. nepalensis (T. Yamaz.) D. Y. Hong, $2x = 16$

Veronicastrum Heist. ex Fabr.
$x = 17$ (8 + 9; see Albach & Chase, 2004)
V. brunonianum (Benth.) D. Y. Hong, $2x_{Vst} = 34$
V. japonicum (Nakai) T. Yamaz., $2x_{Vst} = 34$
V. liukiuense (Ohwi) T. Yamaz., $8x_{Vst} = 136$
V. sibiricum (L.) Pennell, $2x_{Vst} = 34$
V. villosulum (Miq.) T. Yamaz., $4x_{Vst} = 68$
V. virginicum Farw., $2x_{Vst} = 31$

Species not investigated: *Veronicastrum axillare* (Siebold & Zucc.) T. Yamaz., *V. caulopterum* (Hance) T. Yamaz., *V. formosanum* (Masam.) T. Yamaz., *V. kitamurae* (Ohwi) T. Yamaz., *V. latifolium* (Hemsl.) T. Yamaz., *V. longispicatum* (Merr.) T. Yamaz., *V. rhombifolium* (Hand.-Mazz.) P. C. Tsoong, *V. robustum* (Diels) D. Y. Hong, *V. stenostachyum* (Hemsl.) T. Yamaz., *V. tagawae* (Ohwi) T. Yamaz., *V. tubiflorum* (Fisch. & C. A. Mey.) H. Hara, *V. yunnanense* (W. W. Sm.) T. Yamaz.

Picrorhiza Royle ex Benth.
$x = 17$ (8 + 9; see Albach & Chase, 2004)
P. kurrooa Royle ex Benth., $4x_{Pic} = 34$

Species not investigated: *Picrorhiza scrophulariiflora* Pennell, *Neopicrorhiza minima* R. R. Mill.

Veronica L.

I. *Veronica* subgen. *Veronica*

1. *Veronica* sect. *Glandulosae* (Römpp) Albach

1a. *Veronica* subsect. *Glandulosae* (Römpp) Stroh
$x = 8$ (?), 9
V. abyssinica Fresen., $6x = 48$
V. glandulosa subsp. *mannii* (Hook. f.) Elenevsky, $6x = 54$
V. gunae Schweinf. ex Engl., $6x = 54$

2. *Veronica* sect. *Scutellatae* G. Don

2a. *Veronica* subsect. *Scutellatae* Benth.
$x = 9$
V. scutellata L., $2x = 18$

3. *Veronica* sect. *Montanae* (Boriss. ex Elenevsky) Assejeva

3a. *Veronica* subsect. *Canae* (T. Yamaz.) Elenevsky
x = 7, 8, ?
V. cana Wall., ?*x* = 50, 52
V. henryi T. Yamaz., 4*x* = 32
V. miqueliana Nakai, 6*x* = 48
V. robusta (Prain) T. Yamaz., 6*x* = 42

Species not investigated: *Veronica chayuensis* D. Y. Hong, *V. deltigera* Wall. ex Benth., *V. fargesii* Franch., *V. forrestii* Diels, *V. japonensis* Makino, *V. laxissima* D. Y. Hong, *V. longipetiolata* D. Y. Hong, *V. muratae* T. Yamaz., *V. oligosperma* Hayata, *V. piroliformis* Franch., *V. riae* H. J. P. Winkl., *V. sutchuensis* Franch., *V. szechuanica* Batalin, *V. taiwanica* T. Yamaz., *V. tibetica* D. Y. Hong, *V. tsinglingensis* D. Y. Hong, *V. vandellioides* Maxim., *V. yunnanensis* D. Y. Hong.

3b. *Veronica* subsect. *Montanae* Boriss. ex Elenevsky
x = 9
V. montana L., 2*x*, 4*x* = 18, 36

4. *Veronica* sect. *Veronica* L.
4a. *Veronica* subsect. *Alpinae* Benth.
x = 9
V. alpina L., 2*x* = 18
V. bellidioides L., 2*x*, 4*x* = 18, 36
V. copelandii Eastw., 2*x* = 18
V. cusickii A. Gray, 2*x*, 8*x* = 18, 72
V. nipponica Makino, 2*x* = 18
V. nutans Bong., 2*x* = 18
V. stelleri Pall. ex Link, 2*x* = 18
V. wormskjoldii Roem. & Schult., 4*x* = 36

4b. *Veronica* subsect. *Gouani* (Römpp) Stroh
x = 9
V. ponae Gouan, 6*x* = 54

4c. *Veronica* subsect. *Urticifoliae* Boriss. ex Elenevsky
x = 9
V. urticifolia Jacq., 2*x* = 18

4d. *Veronica* subsect. *Veronica*
x = 9
V. allionii Vill., 2*x* = 18
V. officinalis L., 2*x*, 4*x* = 18, 36
V. onoei Franch. & Sav., 4*x* = 36
V. ×*tournefortii* (Vill.) F. W. Schmidt, 3*x* = 27 (= *V. allionii* × *V. officinalis*)

Species not investigated: *Veronica dabneyi* Hochst., *V. morrisonicola* Hayata.

4e. *Veronica* subsect. *Aphyllae* (Römpp) Stroh
x = 9
V. aphylla L., 2*x* = 18
V. grandiflora Gaertn., ?*x* = 48–50

4f. *Veronica* subsect. *Carpathicae* Elenevsky
x = 7
V. baumgartenii Roem. & Schult., 2*x* = 14

II. *Veronica* subgen. *Beccabunga* (Hill) M. M. Mart. Ort., Albach & M. A. Fisch.
Incertae sedis
x = 9
V. hispidula Boiss. & Huet., 2*x*, 4*x* = 18, 36

1. *Veronica* sect. *Beccabunga* (Hill) Dumort.
1a. *Veronica* subsect. *Beccabunga* (Hill) Elenevsky
x = 9
V. americana Schwein. ex Benth., 4*x* = 36
V. beccabunga L. subsp. *beccabunga*, 2*x*, 4*x* = 18, 36
V. beccabunga subsp. *abscondita* M. A. Fisch., 2*x* = 18
V. beccabunga subsp. *muscosa* (Korsh.) Elenevsky, 2*x* = 18

1b. *Veronica* subsect. ined.
x = 9
V. anagallis-aquatica L., 4*x* = 36
V. anagalloides Guss. subsp. *anagalloides*, 2*x*, 4*x* = 18, 36
V. anagalloides subsp. *heureka* M. A. Fisch., 2*x*, 4*x* = 18, 36
V. catenata Pennell, 4*x* = 36
V. lysimachioides Boiss., 2*x* = 18
V. michauxii Lam., 4*x* = 36
V. oxycarpa Boiss., 2*x*, 4*x* = 18, 36
V. poljensis Murb., 4*x* = 36
V. scardica Griseb., 2*x*, 4*x* = 18, 36
V. spec. indet. aff. "beccabungoides," 4*x* = 36
V. undulata Wall., 6*x* = 54
V. ×*myriantha* Tosh. Tanaka, 5*x* = 45
V. anagallis-aquatica × *V. michauxii*, 4*x* = 36

Species not investigated: *Veronica kaiseri* Täckh.

1c. *Veronica* subsect. *Peregrinae* Elenevsky
x = 9
Veronica peregrina L., 6*x* (−2) = 52

2. *Veronica* sect. *Acinifolia* (Römpp) Albach
2a. *Veronica* subsect. *Acinifolia* (Römpp) Stroh
x = 7
V. acinifolia L., 2*x* = 14
V. bozakmanii M. A. Fisch., 4*x* = 28
V. pusilla Kotschy & Boiss., 2*x* = 14
V. reuteriana Boiss., 4*x*, 6*x* = 28, 42
V. syriaca Roem. & Schult., 2*x* = 14

Species not investigated: *Veronica balansae* Stroh, *V. debilis* Freyn, *V. oetaea* Gustavsson, *V. yildirimlii* Öztürk.

3. *Veronica* sect. *Serpyllifolia* G. Don
3a. *Veronica* subsect. *Gentianoides* (G. Don) Assejeva
x = 8 (?)
V. gentianoides Vahl, 2–10*x* = 16, 24, 32, 36, 40, 48, 56, 64, 72, 80
V. schistosa E. Busch, 3*x*, 6*x* = 24, 48
Species not investigated: *Veronica kopgecidiensis* Öztürk & M. A. Fisch.

3b. *Veronica* subsect. *Serpyllifolia* (G. Don) Stroh
x = 7
V. nevadensis (Pau) Pau var. *nevadensis*, 2*x* = 14
V. nevadensis var. *langei* (Lacaita) M. M. Mart. Ort. & E. Rico, 2*x* = 14
V. serpyllifolia L. subsp. *serpyllifolia*, 2*x*, 4*x* = 14, 28
V. serpyllifolia subsp. *repens* (Clarion ex DC.) Hartl in Hegi, 2*x* = 14

Species not investigated: *Veronica archboldii* Pennell, *V. platycarpa* Pennell.

3c. *Veronica* subsect. *Telephiifolia* M. M. Mart. Ort. & E. Rico

$x = 7$

V. telephiifolia Vahl, $2x = 14$

Species not investigated: *Veronica daranica* Saeidi & Ghahr., *V. davisii* M. A. Fisch.

III. *Veronica* subg. *Pseudolysimachium* (Opiz) Buchenau

1. *Veronica* sect. *Schmidtianae* (Boriss. ex. T. Yamaz.) Assejeva

1a. *Veronica* subsect. *Schmidtiana* Boriss. ex Elenevsky

$x_{Psl} = 17$ (9 + 9 − 1)

V. nakaiana Ohwi, $2x = 34$

V. schmidtiana Regel, $2x = 34$

2. *Veronica* sect. *Pseudolysimachium* W. D. J. Koch

2a. *Veronica* subsect. *Alatavicae* Boriss. ex Elenevsky

Species not investigated: *Veronica alatavica* Popov, *V. qingheensis* Y. Z. Zhao.

2b. *Veronica* subsect. *Dahuricae* (Holub) Elenevsky

$x_{Psl} = 17$ (9 + 9 − 1)

V. daurica Steven, $2\ x_{Psl} = 34$ (printing error?)

V. olgensis Kom., $2\ x_{Psl} = 24$

Species not investigated: *Veronica ogurae* (T. Yamaz.) Albach, *V. pyrethrina* Nakai.

2c. *Veronica* subsect. *Pinnatae* (Holub) Elenevsky

$x_{Psl} = 17$ (9 + 9 − 1)

V. pinnata L., $2\ x_{Psl} = 34$

Species not investigated: *Veronica laeta* Kar. & Kir., *V. sessiliflora* Bunge ex Ledeb.

2d. *Veronica* subsect. *Longifoliae* (Holub) Elenevsky

$x_{Psl} = 17$ (9 + 9 − 1)

V. bachofenii Heuff., $2\ x_{Psl} = 34$

V. kiusiana subsp. *maritima* (Nakai) T. Yamaz., $4\ x_{Psl} = 68$

V. kiusiana subsp. *miyabei* (Nakai & Hondo) T. Yamaz., $2\ x_{Psl} = 34$

V. longifolia L., $2\ x_{Psl}$, $4\ x_{Psl} = 34, 68$

V. subsessilis (Miq.) Carriere, $4\ x_{Psl} = 68$

Species not investigated: *Veronica ovata* Nakai, *V. sachalinensis* T. Yamaz., *V. sajanensis* Printz, *V. sieboldiana* Miq., *V. taigischensis* Stepanov.

2e. *Veronica* subsect. *Pseudolysimachium* (W. D. J. Koch) Elenevsky

$x_{Psl} = 17$ (9 + 9 − 1)

V. barrelieri H. Schott ex Roem. & Schult. subsp. *barrelieri*, $2\ x_{Psl}$, $4\ x_{Psl} = 34, 68$

V. barrelieri subsp. *crassifolia* Wierzb., $4\ x_{Psl} = 68$

V. barrelieri subsp. *prodanii* (Degen) Albach, $4\ x_{Psl} = 68$

V. incana L., $4\ x_{Psl} = 68$

V. incana subsp. *incana*, $2\ x_{Psl} = 34$

V. incana subsp. *pallens* (Host) Albach, $4\ x_{Psl} = 68$

V. orchidea Crantz, $2\ x_{Psl}$, $4\ x_{Psl} = 34, 68$

V. ornata Monjuschko, $4\ x_{Psl} = 68$

V. porphyriana Pavlov, $2\ x_{Psl}$ (flow cytometry)

V. spicata L. subsp. *spicata*, $2\ x_{Psl}$, $4\ x_{Psl} = 34, 68$

V. spicata subsp. *euxina* (Turrill) Stoj. & Stef., $2\ x_{Psl} = 34$

V. spicata subsp. *fischeri* Trávn., $4\ x_{Psl} = 68$

V. spicata subsp. *lanisepala* (Trávn.) Albach, $2\ x_{Psl} = 34$

2f. *Veronica* subsect. *Spuriae* (Holub) Elenevsky

$x_{Psl} = 17$ (9 + 9 − 1)

V. linariifolia Pall. ex Link, $2\ x_{Psl} = 34$

V. rotunda Nakai, $2\ x_{Psl} = 34$

V. spuria L., $2\ x_{Psl}$, $4\ x_{Psl} = 34, 68$

IV. *Veronica* subg. *Synthyris* (Benth.) M. M. Mart. Ort., Albach & M. A. Fisch.

$x = 12$

V. alaskensis M. M. Mart. Ort. & Albach, $2x = 24$

V. besseya M. M. Mart. Ort. & Albach, $2x = 24$

V. bullii (Eaton) M. M. Mart. Ort. & Albach, $2x = 24$

V. californica M. M. Mart. Ort. & Albach, $2x = 24$

V. canbyi (Pennell) M. M. Mart. Ort. & Albach, $4x = 48$

V. dissecta (Rydb.) M. M. Mart. Ort. & Albach, $2x = 24$

V. idahoensis M. M. Mart. Ort. & Albach, $2x = 24$

V. missurica Raf. subsp. *missurica*, $2x = 24$

V. missurica subsp. *major* (Hook.) M. M. Mart. Ort. & Albach, $2x$, $4x = 24, 48$

V. missurica subsp. *stellata* (Pennell) M. M. Mart. Ort. & Albach, $2x$, $4x = 24, 48$

V. oblongifolia (Pennell) M. M. Mart. Ort. & Albach, $2x = 24$

V. paysonii (Pennell & L. O. Williams) M. M. Mart. Ort. & Albach, $2x = 24$

V. plantaginea E. James, $2x$, $4x = 24, 48$

V. ranunculina (Pennell) M. M. Mart. Ort. & Albach, $2x = 24$

V. regina-nivalis M. M. Mart. Ort. & Albach, $2x = 24$

V. ritteriana (Eastw.) M. M. Mart. Ort. & Albach, $4x = 48$

V. rubra (Douglas) M. M. Mart. Ort. & Albach, $2x = 24$

V. schizantha (Piper) M. M. Mart. Ort. & Albach, $2x = 24$

V. utahensis M. M. Mart. Ort. & Albach, $2x = 24$

V. wyomingensis (A. Nelson) M. M. Mart. Ort. & Albach, $2x$, $4x = 24, 48$

V. missurica × *V. rubra*, $2x = 24$

V. *Veronica* subg. *Cochlidiosperma* (Rchb.) M. M. Mart. Ort. & Albach

1. *Veronica* sect. *Diplophyllum* (Lehm.) Boriss.

$x = 9$

V. crista-galli Steven, $2x = 18$

Species not investigated: *Veronica simensis* Fresen.

2. *Veronica* sect. *Cochlidiosperma* (Rchb.) Benth.

2a. *Veronica* subsect. *Cymbalariae* Benth.

$x = 9$

V. cymbalaria Bodard, $4x$, $6x = 36, 54$

V. lycica E. B. J. Lehm., $2x = 18$

V. panormitana Tineo, $2x = 18$

V. stamatiadae M. A. Fisch. & Greuter, $2x = 18$

V. trichadena Jord. & Fourr., $2x$, $4x = 18, 36$

2b. *Veronica* subsect. *Cochlidiosperma* (Rchb.) Albach

$x = 9$, ?

V. sibthorpioides Deb., Degen & Hervier, $?x = 28, 30$

V. hederifolia L., $6x = 54$

V. stewartii Pennell, $2x = 18$

V. sublobata M. A. Fisch., $4x = 36$

V. triloba Opiz, $2x = 18$

VI. *Veronica* subg. *Pellidosperma* (E. B. J. Lehm.) M. M. Mart. Ort., Albach & M. A. Fisch.

$x = 7, 8, 9$

V. glauca Sibth. & Sm. subsp. *glauca*, $2x = 18$
V. glauca subsp. *chaubardii* (Boiss. & Reut.) Maire & Petitm., $2x = 18$
V. glauca subsp. *peloponnesiaca* (Boiss. & Orph.) Maire & Petitm., $2x = 18$
V. mazanderanae Wendelbo, $2x = 16$
V. praecox All., $2x = 18$
V. triphyllos L., $2x = 14$

Species not investigated: *Veronica aznavourii* Dörfl., *V. donii* Römpp, *V. samuelssonii* Rech. f.

VII. *Veronica* subg. *Stenocarpon* (Boriss.) M. M. Mart. Ort., Albach & M. A. Fisch.

$x = 8$

V. ciliata Fisch. subsp. *ciliata*, $2x = 16$
V. ciliata subsp. *cephaloides* (Pennell) D. Y. Hong, $2x = 16$
V. contandriopouli Quézel, $4x = 32$
V. densiflora Ledeb., $2x = 16$
V. erinoides Boiss. & Spruner, $2x = 16$
V. fruticans Jacq. subsp. *fruticans*, $2x = 16$
V. fruticans subsp. *cantabrica* M. Laínz, $2x = 16$
V. fruticulosa L., $2x = 16$
V. kellereri Degen & Urum., $2x = 16$
V. mampodrensis Losa & P. Monts., $2x = 16$
V. nummularia Gouan, $2x = 16$
V. saturejoides Vis., $2x = 16$
V. thessalica Benth., $2x = 16$

Species not investigated: *Veronica cachemirica* Gand., *V. capitata* Royle ex Benth., *V. chinoalpina* T. Yamaz., *V. daghestanica* Trautv., *V. emodi* T. Yamaz., *V. eriogyne* H. J. P. Winkl., *V. fedtschenkoi* Boriss., *V. filipes* P. C. Tsoong, *V. gorbunovii* Gontsch., *V. himalensis* D. Don, *V. kotschyana* Benth., *V. lanosa* Royle ex Benth., *V. lanuginosa* Benth., *V. luetkeana* Rupr., *V. macrostemon* Bunge ex Ledeb., *V. macrostemonoides* Zakirov, *V. mexicana* S. Watson, *V. monticola* Trautv., *V. quezelii* M. A. Fisch., *V. rockii* H.-L. Li, *V. ruprechtii* Lipsky, *V. serpylloides* Regel, *V. tianschanica* Lincz.

VIII. *Veronica* subg. *Pocilla* (Dumort.) M. M. Mart. Ort., Albach & M. A. Fisch.

Species not investigated: *Veronica amoena* Steven ex M. Bieb., *V. violifolia* Hochst. ex Benth.

1. *Veronica* sect. *Subracemosae* (Benth.) Assejeva
1a. *Veronica* subsect. *Subracemosae* Benth.

$x = 7$

V. arguteserrata Regel & Schmalh., $6x = 42$
V. biloba L., $4x = 28$
V. campylopoda Boiss., $4x, 6x, 8x = 28, 42, 56$
V. capillipes Nevski, $4x = 28$
V. arguteserrata × *V. campylopoda*, $6x = 42$

Species not investigated: *V. bucharica* B. Fedtsch., *V. nevskii* Boriss., *V. ramosissima* Boriss., *V. stylophora* Popov ex Vved., *V. tenuissima* Boriss.

1b. *Veronica* subsect. *Cardiocarpae* Boriss. ex Elenevsky

$x = 7$ or 8?

V. cardiocarpa (Kar. & Kir.) Walp., $2x = 14, 16$
Species not investigated: *Veronica intercedens* Bornm.

1c. *Veronica* subsect. *Brevistylae* Elenevsky

$x = 7$

V. rubrifolia subsp. *respectatissima* M. A. Fisch., $4x = 28$

Species not investigated: *Veronica avromanica* M. A. Fisch., *V. ferganica* Popov, *V. macropoda* Boiss., *V. viscosa* Boiss.

2. *Veronica* sect. *Pocilla* Dumort.
2a. *Veronica* subsect. *Agrestes* Benth.

$x = 7$

V. agrestis L., $4x = 28$
V. ceratocarpa C. A. Mey., $2x = 14$
V. filiformis Sm., $2x = 14$
V. francispetae M. A. Fisch., $2x = 14$
V. opaca Fr., $4x = 28$
V. persica Poir., $4x = 28$
V. polita Fr., $2x = 14$
V. siaretensis E. B. J. Lehm., $2x = 14$

Species not investigated: *Veronica bungei* Boiss., *V. longipedicellata* Saeidi.

IX. *Veronica* subgen. *Pentasepalae* (Benth.) M. M. Mart. Ort., Albach & M. A. Fisch.

Incertae sedis

Species not investigated: *Veronica aucheri* Boiss., *V. chionantha* Bornm., *V. czerniakowskiana* Monjuschko, *V. gaubae* Bornm., *V. khorassanica* Czerniak., *V. kopetdaghensis* B. Fedtsch., *V. minuta* C. A. Mey., *V. mirabilis* Wendelbo, *V. paederotae* Boiss.

1a. *Veronica* subsect. *Pentasepalae* Benth.

$x = 8$

V. aragonensis Stroh, $4x = 32$
V. austriaca L., $6x, 8x = 48, 64$
V. austriaca subsp. *dostalii* (Domin) Dostal, $6x = 48$
V. crinita Kit. ex Schult., $2x = 16$
V. jacquinii Baumg., $2x, 6x, 8x, 10x = 16, 48, 64, 80$
V. jacquinii subsp. *neicefii* Degen, $4x = 32$
V. kindlii Adamović, $2x = 16$
V. krylovii Schischk., $2x = 16$
V. orbiculata A. Kern, $4x = 32$
V. orsiniana Ten., $2x = 16$
V. prostrata L. subsp. *prostrata*, $2x = 16$
V. prostrata subsp. *sibirica* Watzl, $2x = 16$
V. rhodopea (Velen.) Degen ex Stoj. & Stef., $2x = 16$
V. rosea Desf., $2x = 16$
V. scheereri (J. P. Brandt) Holub, $4x = 32$
V. sennenii (Pau) M. M. Mart. Ort. & E. Rico, $8x = 64$
V. tenuifolia Asso subsp. *tenuifolia*, $2x = 16$
V. tenuifolia subsp. *fontqueri* (Pau) M. M. Mart. Ort. & E. Rico, $2x = 16$
V. tenuifolia subsp. *javalambrensis* (Pau) Molero & A. Pujadas, $2x, 4x = 16, 32$
V. teucrium L., $6x, 8x = 48, 64$
V. turrilliana Stoj. & Stef., $2x = 16$
Veronica ×*gundisalvi* Sennen, $2x = 16$

1b. *Veronica* subsect. *Armeno-persicae* Stroh

$x = 8$

V. armena Boiss. & Huet., $2x = 16$

V. farinosa Hausskn., $2x = 16$
V. microcarpa Boiss., $4x = 32$

Species not investigated: *Veronica acrotheca* Bornm. & Gauba, *V. euphrasiifolia* Link, *V. liwanensis* K. Koch, *V. montbretii*, M. A. Fisch., *V. oltensis* Woronow.

1c. *Veronica* subsect. *Orientales* (Wulff) Stroh
$x = 8$
V. bombycina Boiss. & Kotschy, $2x = 16$
V. caespitosa Boiss., $2x = 16$
V. cinerea Boiss. & Bal., $2x = 16$
V. cuneifolia D. Don, $2x = 16$
V. dichrus Schott & Kotschy, $2x = 16$
V. elmaliensis M. A. Fisch., $8x = 64$
V. kurdica Benth., $6x = 48$
V. macrostachya subsp. *sorgerae* M. A. Fisch., $2x = 16$
V. multifida L., $2x$, $4x$, $6x$, $10x = 16, 32, 48, 80$
V. multifida × *V. dichrus*, $8x = 64$
V. orientalis Mill., $4x$, $8x = 32, 64$
V. pectinata L., $2x = 16$
V. thymifolia Sibth. & Sm., $2x = 16$

Species not investigated: *Veronica allahuekberensis* Öztürk, *V. antalyensis* M. A. Fisch., Erik & Sümbül, *V. cetikiana* Öztürk, *V. fragilis* Boiss. & Hausskn., *V. fridericae* M. A. Fisch., *V. fuhsii* Freyn & Sint., *V. galathica* Boiss., *V. leiocarpa* Boiss., *V. polifolia* Benth., *V. polium* P. H. Davis, *V. rechingeri* M. A. Fisch., *V. surculosa* Boiss. & Balansa, *V. taurica* Willd., *V. tauricola* Bornm., *V. thymoides* P. H. Davis.

1d. *Veronica* subsect. *Petraea* Benth.
$x = 8$
V. peduncularis M. Bieb., $2x = 16$

Species not investigated: *Veronica baranetzkii* Bordz., *V. bogosensis* Tumadz., *V. borisovae* Holub, *V. caucasica* M. Bieb., *V. filifolia* Lipsky, *V. petraea* (M. Bieb.) Steven, *V. umbrosa* M. Bieb., *V. vendetta-deae* Albach.

X. *Veronica* subg. *Chamaedrys* (W. D. J. Koch) Buchenau

1. *Veronica* sect. *Alsinebe* Grisb.
1a. *Veronica* subsect. *Microspermae* (Römpp) Stroh
$x = 8$
V. arvensis L., $2x = 16$

Species not investigated: *Veronica sartoriana* Boiss. & Heldr.

1b. *Veronica* subsect. *Microspermoides* Albach
$x = 8$
V. brevistyla Moris, $2x = 16$
V. dillenii Crantz, $2x = 16$
V. verna L., $2x = 16$

2. *Veronica* sect. *Chamaedrys* W. D. J. Koch

2a. *Veronica* subsect. *Asiachamaedrys* Albach
$x = 7?, ?$
V. laxa Benth., $4x$, $?x = 32, 46$
V. magna M. A. Fisch., $6x = 42$

2b. *Veronica* subsect. *Multiflorae* Benth.
$x = 8$
V. chamaedryoides Bory & Chaub., $2x = 16$
V. chamaedrys L. subsp. *chamaedrys*, $2x$, $4x = 16, 32$
V. chamaedrys subsp. *micans* M. A. Fisch., $2x = 16$
V. krumovii (Peev) Peev, $2x = 16$
V. micrantha Hoffmanns. & Link, $2x = 16$
V. orbelica (D. Peev) D. Peev, $2x = 16$
V. vindobonensis (M. A. Fisch.) M. A. Fisch., $2x = 16$

XI. *Veronica* subgen. *Pseudoveronica* J. B. Armstr.

1. *Veronica* sect. *Detzneria* (Schltr. ex Diels) Albach
$x = ?$
V. tubata (Diels) Albach, $6x = 38$–42 or 48

2. *Veronica* sect. *Derwentia* (Raf.) B. G. Briggs
$x_{Hebe} = 19, 20, 21$
24 species, see Briggs and Ehrendorfer (2006) for review.

3. *Veronica* sect. *Hebe* (Juss.) Benth.
$x_{Hebe} = 20, 21$
For 121 species from New Zealand and adjacent islands, see Bayly and Kellow (2006) and Garnock-Jones and Lloyd (2004) for a review.

Species from New Guinea:
V. albiflora (Pennell) Albach, $2\ x_{Hebe} = 12$
V. ionantha Albach, $2\ x_{Hebe} = 40$

Species not investigated: *Veronica brassii* (Pennell) Albach, *V. carminea* Albach, *V. carstensensis* Wernham, *V. diosmoides* Schltr., *V. inflexa* Albach, *V. papuana* (P. Royen & Ehrend.) Albach, *V. strigosa* Albach, *V. vandeveateri* Wernham, *V. wilhelminensis* Albach.

XII. *Veronica* subg. *Triangulicapsula* M. M. Mart. Ort., Albach & M. A. Fisch.
$x = 6$
V. chamaepithyoides Lam., $4x = 24$
V. grisebachii Walters, $2x = 12$

Incertae Sedis within *Veronica*
$x = 8$
V. javanica Blume, $2x = 16$

TOWARD A PHYLOGENY OF *MIMOSA* (LEGUMINOSAE: MIMOSOIDAE): A PRELIMINARY ANALYSIS OF SOUTHERN SOUTH AMERICAN SPECIES BASED ON CHLOROPLAST DNA SEQUENCE[1]

C. Bessega,[2] H. E. Hopp,[3] and R. H. Fortunato[4]

Abstract

The pantropical and subpantropical genus *Mimosa* L. comprises more than 500 species, of which nearly 480 are reported for the American Continent. *Mimosa* is subdivided into five sections, four of which are represented in southern South America: *Batocaulon* DC., *Habbasia* DC., *Calothamnos* Barneby, and *Mimosa*. Previous taxonomic studies of the species from the austral region have found classification conflicts among (a) sections *Batocaulon* and *Habbasia*, (b) sections *Calothamnos* and *Mimosa*, and (c) series and subseries within section *Mimosa*. This paper reports a preliminary phylogenetic analysis of chloroplast nucleotide sequences of the *trnL* intron and the *trnL-trnF* intergenic spacer from 34 species of *Mimosa* and related genera. Key morphological characters were mapped onto the phylogenetic hypothesis and discussed. Sequence analysis indicates that the genus *Mimosa* is monophyletic; it is derived from *Piptadenia viridiflora* (Kunth) Benth. The four sections proposed by Barneby are not natural groups. The cladogram retrieved indicates that the representatives of *Mimosa* sect. *Batocaulon* are not clustered, the xerophylous representatives of this section are basal, and the remaining species are related to those species of section *Habbasia*, suggesting that section *Batocaulon* ser. *Stipellares* is more recently derived. The species of *Mimosa* sect. *Calothamnos* that were analyzed are nested in section *Mimosa*. The results seem to support retention of this section within section *Mimosa* as was noted previously by Bentham. The chloroplast sequence data suggest that the representatives from sections *Calothamnos* and *Mimosa* share a common ancestor with those from section *Habbasia* and section *Batocaulon* ser. *Stipellares*.

Key words: cpDNA, *Mimosa*, phylogeny, *trnL* intron, *trnL-trnF* intergenic spacer.

The pantropical and subpantropical genus *Mimosa* L. has about 500 species. Nearly 480 are reported for the American Continent (Barneby, 1991, 1993; Turner 1994a, b; Fortunato & Palese, 1999; Queiroz et al., 1999; Grether, 2000; Silva & Secco, 2000; Izaguirre & Beyhaut, 2002). The first comprehensive taxonomic revision of this diverse genus was proposed by Bentham (1875) in his global monograph of the Mimoseae and later supplemented in *Flora Brasiliensis* (1876). Bentham (1841–1842, 1875) recognized in the genus: (a) section *Mimosa*, with haplostemonous flowers; and (b) *Habbasia* DC., with diplostemonous flowers, each organized into infrasectional ranks. The generic definition of *Mimosa* as proposed by Bentham has found nearly universal acceptance. Its limits were only challenged by Britton and Rose (1928) in *North American Flora*, with the introduction of a novel division. They segregated *Mimosa* as another genera based on species that had different pod type and leaf reduction (= phyllodes). However, their artificial classification has largely been ignored. In South America, the only revision of the Argentinean species was published by Burkart (1948). His treatment was largely in accord with Bentham's infrageneric classi-

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fication, even though eight additional species and seven varieties were described.

Although there has been no global evaluation of *Mimosa* since Bentham (1875, 1876), Barneby (1991) presented a new taxonomic treatment of *Mimosa* in the New World that makes up approximately 90% of the total species. He noted two chief foci of speciation in the Americas: (a) Brazil south of Amazonia and adjacent areas of Paraguay, Argentina, and Uruguay; and (b) central and southern Mexico, as well as other minor ones in Cuba-Hispaniola, and the Orinoco basin. In addition to this, Barneby expanded Bentham's generic definition, but he also included the genera *Schrankia* Willd. and *Schranckiastrum* Hassl. He reorganized the sectional and infrasectional classification, established new categories, and reordered the old ones. This classification recognized five sections in *Mimosa*: *Mimadenia* Barneby, *Batocaulon* DC., *Habbasia*, *Calothamnos* Barneby, and *Mimosa* (Table 1).

In the phylogeny proposed by Barneby, *Mimosa* was derived from Piptadenioid ancestors (Barneby, 1991: 15–16). He suggested that section *Mimadenia* is a morphological group intermediate between the Piptadenioid ancestor and the remaining species of *Mimosa*. The novel position he gives to section *Mimadenia* is based on the extra floral nectaries that resemble both genera, and the ovate anthers that are intermediate between the orbicular connective, girdled by incurved anther sacs of *Mimosa* and the oblong connective, which is flanked by straight parallel anther sacs of *Piptadenia* Benth. The anthers of the species of *Mimosa* sect. *Mimadenia* always lack the terminal gland present in *Adenopodia* C. Presl and *Piptadenia*. The craspedium type of some species of *Mimosa* sect. *Mimadenia* is not different from that of *Adenopodia* and *Entada* Adans., but is not related to the valvate pod present in *Piptadenia* s.l. (Barneby, 1991: 25).

All diplostemonous species of *Mimosa* were included in section *Habbasia* and divided by Bentham (1875) into two groups: (1) those with setose pubescence and (2) those with less frequent setose pubescence (Table 1). However, in Barneby's classification (1991), only the first group was maintained in this section. The other group was recognized as section *Batocaulon*. Further, Barneby (1991: 24) proposed that section *Batocaulon* is a derivative of *Mimadenia*. Bentham's concept of section *Batocaulon* was mainly based on the presence of cauline setae and the setiform cilia on leaflet margins, instead of stamen number, which he formerly used to characterize both sections *Mimosa* and *Habbasia*. It is important to mention that there are exceptions in the species admitted by Barneby in *Batocaulon* and now, with his definition, it has become the most heterogeneous section of the genus.

Barneby's sections *Calothamnos* and *Habbasia* were postulated to be derived from section *Batocaulon*. Section *Calothamnos* (sect. *Mimosa* ser. *Lepidotae* Benth. [Table 1]) was defined by Barneby (1991) as an independent sectional rank on the basis of the stellate setae. He proposed that the stellate seta type was clearly derived from the plumose one by shortening of the primary axis and elimination of some or all of the branches. According to Barneby's position (1991), the survival of the rudimentary inner set of filaments in some species of *Calothamnos* suggests that the origin of the section could be closer to the diplostemonous and stellate flowers from section *Batocaulon* ser. *Leiocarpae*.

Although *Mimosa lanuginosa* Glaz. ex Burkart and *M. diversipila* Micheli have stellate seta indumentum, in Barneby's taxonomic classification (1991), they were excluded from section *Calothamnos* and transferred to section *Mimosa* ser. *Mimosa* subser. *Polycephalae* (Benth.) Barneby and section *Mimosa* ser. *Mimosa* subser. *Brevipedes* (Benth.) Barneby, respectively. *Mimosa lanuginosa* shares habit type and the paleaceous calyx with other members of subseries *Polycephalae*, although both features are unknown in section *Calothamnos*. In contrast, the calyx of *M. diversipila* is reduced to a rim as is the case in many species of section *Calothamnos*, but it is a virgate subshrub with mostly simple, densely foliate stems extending into long efoliate racemes of capitula, a condition absent from other members of section *Calothamnos*. Based on Barneby's proposal, the plumose indumenta present in several infrageneric groups could be considered a convergence in an evolutionary scenario.

The haplostemonous section *Mimosa* was considered to be derived from *Habbasia*. Barneby (1991) excluded series *Lepidotae* and *Spiciflorae* Benth. from section *Mimosa* (Table 1). The *Spiciflorae* species were transferred to *Batocaulon* ser. *Plurijugate* Karsten, although they have either a tetramerous or pentamerous perianth and lack the setiform trichomes characteristic of section *Mimosa* (Barneby, 1991). Section *Mimosa* sensu Bentham (1875), or Barneby (1991), shows the most derived features such as the habit (mainly subshrubs to herbs), reduction of leaf formula, craspedodrome leaflet venation, and reduction of petals and androecium numbers (Barneby, 1991: 23).

Four of the five sections recognized by Barneby (1991) are represented in southern South America: *Batocaulon*, *Habbasia*, *Calothamnos*, and *Mimosa*. During the taxonomic analysis of the species from that austral region (Fortunato, unpublished data), conflicts of delimitation were found between: (a) sections *Batocaulon* and *Habbasia*, (b) sections *Calothamnos* and *Mimosa*, and (c) among series and subseries within section *Mimosa*.

Thus, Barneby's hierarchical treatment of *Mimosa* has raised some controversial issues. In an effort to examine these, we propose to use information from genomic data, which are still lacking for *Mimosa* species. Noncoding chloroplast DNA (cpDNA) regions have been used extensively for plant phylogenetic analyses and have proven to be particularly informative at the infrageneric level or when comparing related genera (Taberlet et al., 1991; Bohle et al., 1994; Gielly & Taberlet, 1994; Sang et al., 1997; Baker et al., 1999; Murphy et al., 2000; Hughes et al., 2003; Luckow et al., 2003, 2005; Simpson et al., 2004; Jobson & Lukow, 2007).

This paper reports preliminary phylogenetic analyses of chloroplast nucleotide sequences of the *trnL* intron and the *trnL-trnF* intergenic spacer from 34 species of *Mimosa* and related genera. The main purposes of this study are to: (1) examine the evolutionary relationships within *Mimosa* and among *Mimosa* and other members of the Leguminosae; (2) determine if *Mimosa* is a monophyletic group and, in the case that it is, what the monophyletic units within the genus are; (3) determine whether the phylogeny based on *trnL* intron and the *trnL-trn*F intergenic spacer data is congruent with the current classification and the evolutionary sequence proposed (Barneby, 1991); and (4) gain further insight into the subsequent phylogenetic studies of the genus *Mimosa*.

Materials and Methods

The *trnL* intron and *trnL-trnF* intergenic spacer regions (Taberlet et al., 1991) were sequenced for a total of 34 taxa (Table 2). We included species that represent sections *Mimosa*, *Habbasia*, *Calothamnos*, and *Batocaulon* distributed in Argentina, Paraguay, and Brazil. We analyzed both fresh leaflets that were collected and dried in the field with silica gel, and leaflets from herbarium specimens. Morphological observations for the species were made at the Instituto de Recursos Biológicos, Instituto Nacional de Tecnología Agropecuraria (INTA), Castelar (BAB) on herbarium loans from B, BA, BAA, BAF, BH, BM, CORD, CTES, E, F, G, GH, HB, K, LIL, M, MO, NY, P, PY, RB, SI, SP, US, and W. Six outgroups representing different mimosoid tribes were selected on the basis of morphological and molecular criteria (Luckow et al., 2003, 2005; Jobson & Luckow, 2007). Taxa, voucher details, and GenBank accession numbers are listed in Table 2.

Genomic DNA was isolated with a CTAB protocol (Doyle & Doyle, 1987) or with the DNA Easy Extraction kit (Qiagen, Valencia, California, U.S.A.) according to the manufacturer's instructions. Two cpDNA regions were amplified from purified DNA via polymerase chain reaction (PCR) using the four primers, c (5′-CGAAATCGGTAGACGCTACG-3′), d (5′-GGGGATAGAGGGACTTGAAC-3′), e (5′-GGTT CAAGTCCCTCTATCCC-3′), and f (5′-ATTTGAA CTGGTGACACGAG-3′) (Taberlet et al., 1991). PCR reactions were of 50 µl volume, including 2.5 units of Taq-DNA polymerase (Invitrogen, Carlsbad, California, U.S.A.), 0.2 mM each dNTP (Invitrogen), 50 ng each primer (synthesized by AlphaDNA, Montreal, Canada), 3 mM $MgCl_2$ (Invitrogen), and 50 ng genomic DNA. Amplification was performed on a Biometra (Goettingen, Germany) T-gradient thermal cycler using the following steps: 95°C for 4 min., 35 cycles of 94°C for 30 sec., 55°C for 30 sec., and 72°C for 2 min., with an additional final extension step of 10 min. at 72°C. Amplified products were separated on 1% agarose Tris-acetate-ethylenediamine tetraacetic acid (TAE) gels and purified using QIAquick Gel Extraction kit (Qiagen) and used as a template for direct sequencing. In some cases, direct sequencing failed and cloning was performed using the pGEM T-Easy Vector System I (Promega, Madison, Wisconsin, U.S.A.) with DH5α competent bacterial strain. Sequencing reactions were performed with an ABI 3730 XL automatic DNA sequencer by Macrogen Inc. (Seoul, Korea). The same primers or the universal T7 and SP6 primers were used to make the primer extension for the direct sequence or cloning fragments, respectively.

Sequences were edited with the computer program BioEdit (Hall, 1999), initial alignment was performed using ClustalX version 1.8 (Thompson et al., 1997) with the default parameters, and minimal manual adjustment was made. Both DNA regions were merged in WinClada (Nixon, 1999). Uncertain positions located near the primer annealing sites and regions in which the alignment was ambiguous were excluded from the data set.

Parsimony analysis was conducted with NONA (Goloboff, 1998) included in the WinClada software (Nixon, 1999) using 1000 random addition sequences, tree bisection-reconnection (TBR) holding 100 trees per replicate, and attempting to swap to completion. Characters were considered unordered and equally weighed. The bootstrap analysis used 1000 replications each with 10 random additions holding 10 in each replicate, with a maximum of 100 trees saved per replication and mapped to the selected tree. Based on principal morphological criteria for delimitation of sections, 10 morphological characters were selected for optimization on the *trnL* intron and the *trnL-trnF* intergenic spacer phylogeny to allow for discussion of the evolution of these characters in a phylogenetic context. Morphological features (listed in Fig. 3) were scored from specimens in the collection of the BAB herbarium. Nine of these characters have two states and one is multistate.

Table 1. Bentham (1875) and Barneby (1991) taxonomic proposal in *Mimosa* L.

Bentham (1875)		Barneby (1991)		
Section	Series	Section	Series	Subseries
Habbasia DC. Setose pubescence		*Habbasia* DC., ca. 80	*Bipinnatae* DC.	
			Habbasia	
			Pachycarpae Benth.	
			Setosae Barneby	
			Neptunioideae Barneby	
			Rondonianeae Barneby	
			Rojasianae Barneby	
			Pseudocymosae Hass	
			Piresianae Barneby	
Occasional setose pubescence	*Glanduliferae* Benth.	*Mimadenia* Barneby, ca. 16	*Glanduliferae* Benth.	
			Nothacaciae Barneby	
			Revolutae Barneby	
	Rubicaules Benth.	*Batocaulon* DC., ca. 85	*Rubicaules* Benth.	
	Acanthocarpae Benth.		*Acanthocarphae* Benth.	
	Ephedroideae Benth.		*Ephedroideae* Benth.	
	Stipellares Benth.		*Stipellares* Benth.	
	Leptopodae Benth. (= Paucifoliatae Benth.)		*Paucifoliate* Benth.	
	Cesalpiniifoliae Benth.		*Cesalpiniifoliae* Benth.	
	Leptostachyae Benth. (= *Leiocarpae* Benth., *Stachyomima* Benth., *Tomentosae* Benth. sensu Bentham, 1841–1842)		*Leiocarpae* Benth.	
			Distachyae Barneby (= Leiocarpae Benth.; Grether, 2000)	
			Andinae Barneby	
			Acantholobae Barneby	
			Boreales Barneby	
			Bimucronatae Barneby	
			Eucaenioideae Barneby	
			Fagaracanthae Barneby	
			Bahamenses Barneby	
			Farinosae Barneby	
			Echinocaulae Barneby	
			Glandulosae (Benth.) Barneby	
			Auriculate Barneby	
			Ceratoniae Barneby	
			Cordistipulae Barneby	
			Campicolae Barneby	
			Filipides Barneby	
			Quadrivalves Barneby	
			Plurijugae Karsten	
Mimosa	*Lepidotae* Benth.	*Calothamnos* Barneby, ca. 24		
	Myriophyllae Benth.	*Mimosa*, ca. 180	*Myriophyllae* Benth.	
	Sensitivae Benth.		*Mimosa*	
	Pectinatae Benth.			*Pectinatae* (Benth.) Barneby
	Pudicae Benth.			*Pudicae* (Benth.) Barneby
	Pedunculosae Benth.			*Pedunculosae* (Benth.) Barneby
	Meticulosae Benth.			*Dolentes* Barneby
				Polycephalae (Benth.) Barneby
	Hirsutae Benth.			*Hirsutae* (Benth.) Barneby
	Obstrigosae Benth.			*Obstrigosae* (Benth.) Barneby

Table 1. Continued.

Bentham (1875)		Barneby (1991)		
Section	Series	Section	Series	Subseries
	Castae Benth.	*Mimosa*, ca. 180		*Castae* (Benth.) Barneby
				Dolentes Barneby
				Polycarpae Barneby
				Teledactylae Barneby (= ser. *Teledactylae* (Barneby) R. Grether; Grether, 2000)
				Lactifluae Barneby (= ser. *Lactifluae* (Barneby) R. Grether; Grether, 2000)
				Bolivianae Barneby
				Lundianae Barneby
				Trichocephalae Barneby
				Ramosissimae Barneby
				Serrae Barneby
				Sparsae Barneby
				Morongianae Barneby
				Bipennatulae Barneby
				Affines Barneby
				Simplicissimae Barneby
				Dicerasteae Barneby
				Discobolae Barneby
				Brevipedes Barneby
				Thomistae Barneby
				Pogocephalae Barneby
				Microcarpae Barneby
				Lanatae Barneby
	Modestae Benth.		*Modestae* Benth.	*Macrocalycinae* Barneby
				Reptantes Barneby
				Ramnetaceae Barneby
				Axillares Barneby
				Dryandroideae Barneby
				Dutranae Barneby
				Widgrenianae Barneby
				Diffusae Barneby
	Spiciflorae Benth.	*Mimadenia* Barneby	*Myriadeniae* Barneby	
		Batocaulon DC.	*Plurijugae* Karsten	

Results

The aligned length for the *trnL* and the *trnL-trnF* region is 633 bp and 514 bp, respectively. In the ingroup, DNA sequence lengths of the intron ranged from 525 bp in *Mimosa paupera* Benth. to 541 bp in *M. somnians* Humb. & Bonpl. ex Willd. The outgroup species had shorter sequences (496–524 bp). For the *trnL-trnF* intergenic spacer, DNA sequence lengths varied from 358 bp in *M. oligophylla* Micheli and *M. balansae* Micheli to 388 bp in *M. paupera*.

A matrix of 582 bp length for the *trnL* intron and 458 bp for the *trnL-trnF* intergenic spacer was analyzed. Gaps in the ingroup varied from 1 to 17 bp in length for the intron and from 1 to 36 bp for the intergenic spacer. The data matrix that was used consisted of 1040 positions, from which 124 (11.9%) were potentially informative characters. Sequence characteristics are summarized in Table 3.

Parsimony analysis resulted in 92 trees consisting of 470 steps with a consistency index (CI) of 0.68 and a retention index (RI) of 0.65, excluding uninformative characters. The majority consensus tree and one of the most parsimonious trees are shown in Figures 1 and 2, respectively. In general terms, the preferred topology is in agreement with the consensus tree. The species of genus *Mimosa* analyzed here constitute a single clade in the majority consensus tree (Fig. 1) and in the preferred topology cladogram (Fig. 2, BS =

Table 2. Species names for DNA used in the present study of *trnL* and *trnL-trnF* sequences, vouchers (deposited at BAB [Herbarium Inst. Recursos Biológicos, INTA, Argentina]), and GenBank accession numbers. Taxa are in alphabetical order. RHF = Renée H. Fortunato; JAG = Julián A. Greppi; MAL = Melissa A. Luckow; EMZ = Elsa M. Zardini.

	Species	Herbarium voucher	*trnL* GenBank numbers	*trnL-trnF* GenBank numbers
	Mimosa adenotricha Benth.	RHF 8450	DQ344579	DQ344613
	Mimosa balansae Micheli	RHF 7534	DQ344552	DQ344585
	Mimosa bifurca Benth. var. *bifurca*	RHF 7556	DQ344553	DQ344586
	Mimosa bonplandii (Gillies ex Hook. & Arn.) Benth.	JAG 92	DQ344581	DQ344615
	Mimosa brevipetiolata Burkart	JAG 127	DQ344582	DQ344616
	Mimosa candollei R. Grether	RHF 7555	DQ344555	DQ344588
	Mimosa debilis Humb. & Bonpl. ex Willd.	RHF 8085	DQ344561	DQ344594
	Mimosa detinens Benth.	MAL 4491	DQ344558	DQ344591
	Mimosa diversipila Micheli var. *diversipila*	RHF 8810	DQ344584	DQ344618
	Mimosa flagellaris Benth.	RHF 7887	DQ344557	DQ344590
	Mimosa hexandra Micheli	MAL 4584	DQ344556	DQ344589
	Mimosa hirsutissima Mart.	RHF 7962	DQ344562	DQ344595
	Mimosa leimonias Barneby & Fortunato	EMZ 41158	DQ344575	DQ344609
	Mimosa maguirei Barneby	RHF 8418	DQ344576	DQ344610
	Mimosa obstrigosa Burkart	RHF 8077	DQ344568	DQ344601
	Mimosa oligophylla Micheli	RHF 8074	DQ344574	DQ344608
	Mimosa paupera Benth.	RHF 8018	DQ344565	DQ344598
	Mimosa pigra L.	JAG 38	DQ344560	DQ344593
	Mimosa pilulifera Benth.	JAG 39	DQ344573	DQ344607
	Mimosa polycarpa Kunth	RHF 8019	DQ344566	DQ344599
	Mimosa radula Benth.	RHF 8413	DQ344577	DQ344611
	Mimosa sensibilis Griseb.	RHF 8385	DQ344580	DQ344614
	Mimosa setosa Benth. var. *setosa*	RHF 8445	DQ344578	DQ344612
	Mimosa somnians Humb. & Bonpl. ex Willd.	RHF 7953	DQ344563	DQ344596
	Mimosa strigillosa Torr. & A. Gray	RHF 8949	DQ344567	DQ344600
	Mimosa tweedieana Barneby ex Glazier & Mackinder	RHF 7949	DQ344572	DQ344606
	Mimosa uliginosa Chodat & Hassl.	RHF 8059	DQ344564	DQ344597
	Mimosa xanthocentra Mart. var. *xanthocentra*	RHF 7650	DQ344559	DQ344592
Outgroup taxa	*Anadenanthera colubrina* (Vell.) Brenan var. *cebil* (Griseb.) Altschul	RHF 7583	DQ344571	DQ344605
	Calliandra tweediei Benth.	RHF 8853	DQ344583	DQ344617
	Mimozyganthus carinatus (Griseb.) Burkart	RHF 7567	DQ344570	DQ344604
	Neptunia pubescens Benth.	RHF 7923	DQ344551	DQ344603
	Parapiptadenia excelsa (Griseb.) Burkart	RHF 7669	DQ344569	DQ344602
	Piptadenia viridiflora (Kunth) Benth.	RHF 7586	DQ344554	DQ344587

80%), indicating that this group is monophyletic. Both cladograms indicate that *Piptadenia viridiflora* (Kunth) Benth. is the species most closely related to *Mimosa* (Fig. 2, BS = 87%).

In both trees, the species of section *Batocaulon* are not clustered together, and *Mimosa hexandra* Micheli and *M. detinens* Benth. are separated from the rest and constitute a basal clade (Fig. 2, BS = 94%). The

Table 3. Sequence characteristics for the *trnL* intron and the *trnL-trnF* intergenic spacer sequenced in this study.

	trnL intron	*trnL-trnF* intergenic spacer	Total
Length range (bp)	496–541	358–388	—
Aligned length (bp)	633	514	1147
No. of sites excluded (bp)	51	56	107
Analyzed region (bp)	582	458	1040
Indels	27	26	53
Size of indels (ingroup) (bp)	1–17	1–36	1–36
Base substitutions	31	40	71
Total potentially informative characters	58	66	124

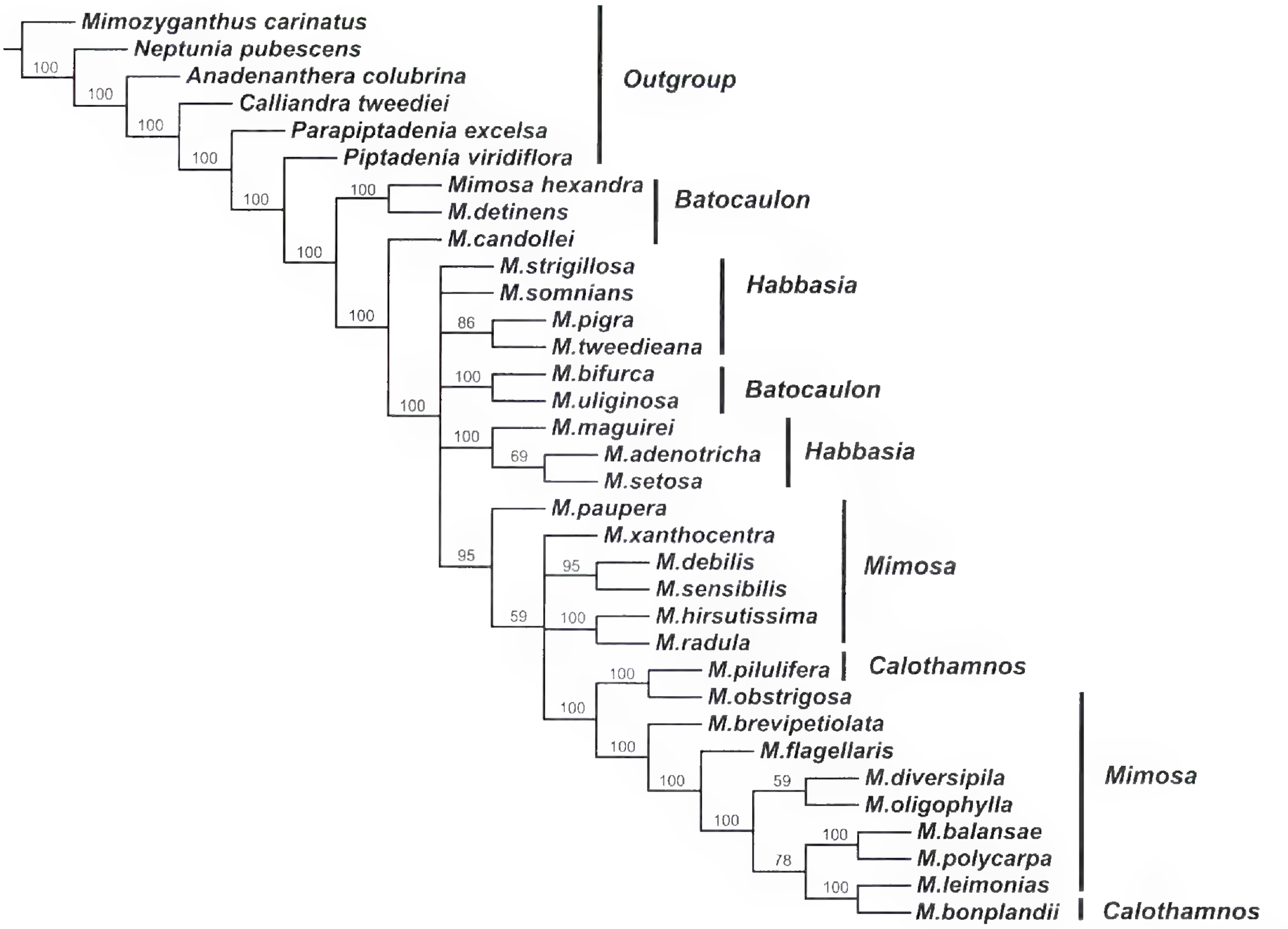


Figure 1. Majority consensus tree obtained from 92 equally parsimonious trees (length 470, CI = 0.68, RI = 0.65) based on *trnL* intron and *trnL-trnF* intergenic spacer sequence data. Numbers above branches are the percentage of trees in which the clade is supported. The sections of the genus are indicated according to Barneby (1991).

remaining species of section *Batocaulon*, *M. uliginosa* Chodat & Hassl., and *M. bifurca* Benth., are placed with the species of section *Habbasia*. From the polymorphic section *Batocaulon*, *M. candollei* R. Grether shares more characters (Fig. 2, BS = 80%) with section *Habbasia* than do *M. hexandra* and *M. detinens*. In Figure 2, *M. bifurca* and *M. uliginosa* are more recently differentiated and clustered with high bootstrap support within section *Habbasia* (Fig. 2, BS = 99%). However, this last clade is not highly supported (Fig. 2, BS < 50%) and can partially explain the polytomy found in the majority consensus tree (Fig. 1). *Mimosa maguirei* Barneby, *M. adenotricha* Benth., and *M. setosa* Benth. (sect. *Habbasia* ser. *Pachycarpae* Benth.) are clustered (Figs. 1, 2, BS = 94%), and the relation among clades integrated by representatives from *Batocaulon* and *Habbasia* is not solved (Fig. 1). On the other hand, the preferred topology (Fig. 2) indicates that the *M. maguirei*, *M. adenotricha*, and *M. setosa* clade is sister in relation to the cluster constituted by *M. tweedieana* Barneby ex Glazier & Mackinder and *M. pigra* L. (sect. *Habbasia* ser. *Habbasia*), *M. strigillosa* Torr. & A. Gray (sect. *Habbasia* ser. *Habbasia*), and *M. somnians* (sect. *Habbasia* ser. *Bipinnatae*) (Fig. 2).

In both figures, the species of sections *Mimosa* and *Calothamnos* constitute a single clade. In Figure 1, *Mimosa paupera* is sister to the remaining species; however, in the consensus tree (Fig. 2), this species is sister to the clade formed by *M. hirsutissima* Mart. and *M. radula* Benth. In a similar way, *M. xanthocentra* Mart. is sister (Fig. 2, BS < 50%) to the clade constituted by *M. debilis* Humb. & Bonpl. ex Willd. and *M. sensibilis* Griseb. (Fig. 2, BS = 71%). In the consensus tree, *M. xanthocentra*, the cluster formed by *M. debilis* and *M. sensibilis*, the cluster formed by *M. hirsutissima* and *M. radula*, and the group that includes the remaining species from sections *Mimosa* and *Calothamnos* that were analyzed constitute a polytomy. A cluster formed by *M. pilulifera* Benth. (sect. *Calothamnos*) and *M. obstrigosa* Burkart (sect. *Mimosa*) can be seen in both trees. From the remaining species, the same topology is found in both trees (Figs. 1, 2). *Mimosa brevipetiolata* Burkart is basal (Fig. 2, BS = 71%), followed by *M. flagellaris* Benth. (Fig. 2, BS = 79%), and a group that is split into two clades (Fig. 2, BS = 72%). The first one (weakly supported) consisted of *M. diversipila* and *M. oligophylla*. The second is a clade (Fig. 2, BS = 50%) formed by two groups; the

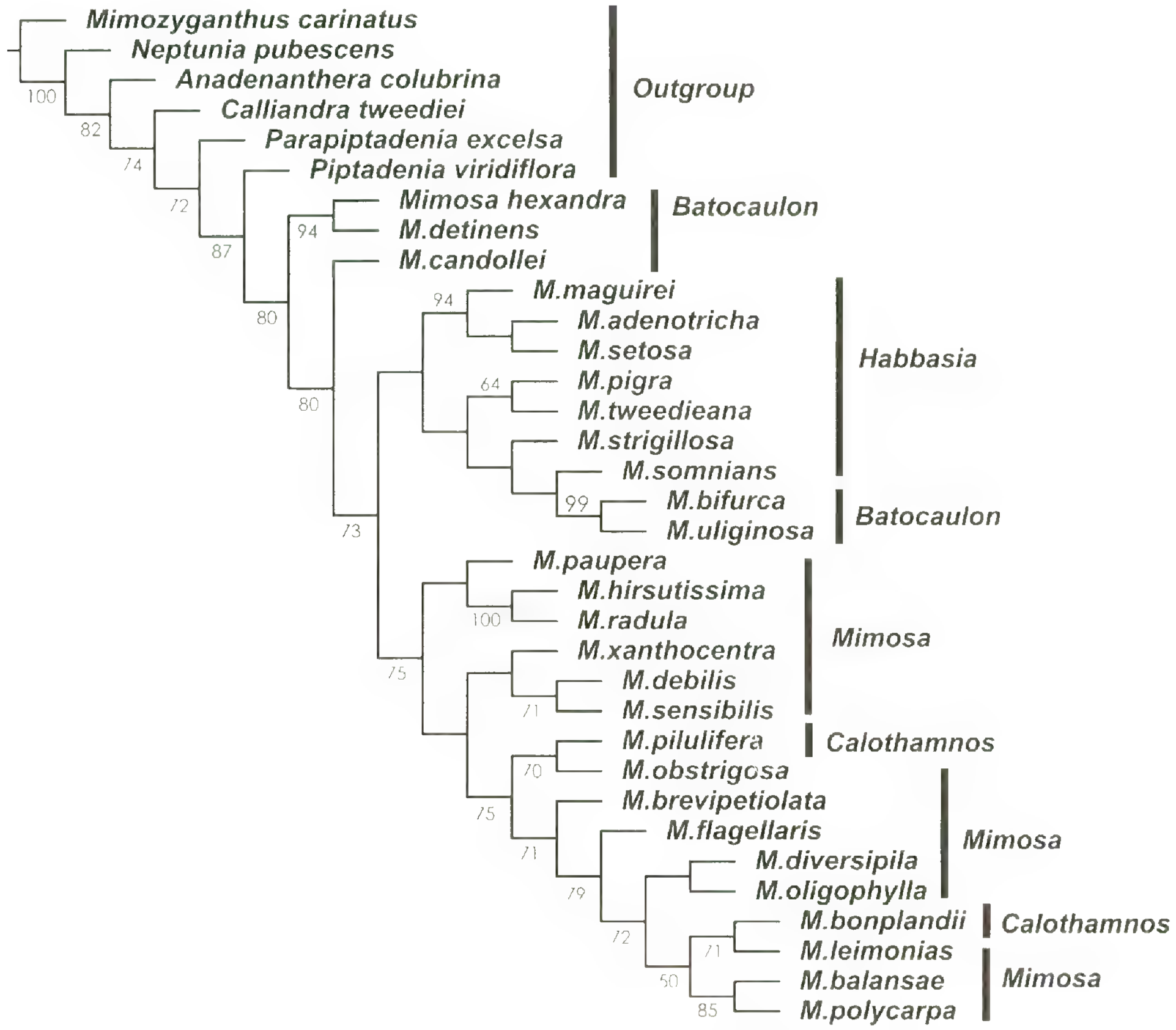


Figure 2. One of the most parsimonious trees of 470 steps based on *trnL* intron and *trnL-trnF* intergenic spacer sequence data. Bootstrap values greater than 50% are shown at the nodes. The sections of the genus are indicated according to Barneby (1991).

first one (Fig. 2, BS = 71%) includes *M. bonplandii* (Gillies ex Hook. & Arn.) Benth. (sect. *Calothamnos*) and *M. leimonias* Barneby & Fortunato (sect. *Mimosa*), and the second includes *M. balansae* and *M. polycarpa* Kunth (Fig. 2, BS = 85%), both from section *Mimosa*.

Discussion

The present study represents the first molecular approach toward a phylogeny of *Mimosa*. This is a preliminary reconstruction based on the *trnL* intron and *trnL-trnF* intergenic spacer, including species from southern South America. Although sampling of taxa is limited, the four sections found in the southern center of diversity of the genus (south of Amazonia in Brazil and adjacent areas of Paraguay, Argentina, and Uruguay) (Barneby, 1991) are represented. Noncoding regions have been presumed to be more useful at lower taxonomic ranks because they are less functionally constrained and are free to vary, thereby potentially providing more phylogenetically informative characters per unit of sequencing effort (Clegg et al., 1994; Gonzalez & Vovides, 2002). In *Mimosa*, both the *trnL* intron and the *trnL-trnF* intergenic spacer exhibited a similar level of informative characters (12% on average) to most of the other plant species assayed with comparable methods (Baker et al., 1999; Bayer et al., 2000; Murphy et al., 2000; Gonzalez & Vovides, 2002). Despite the fact that the ambiguous regions for the alignments were excluded, a large data set (1040 characters) was obtained, and 124 informative sites could be retrieved for the sampled species. A low level of conflict among data ensured that many of the relationships recovered were highly supported and are addressed in this contribution.

The cpDNA allowed us to make a phylogenetic hypothesis. The cladogram indicates that the four sections of *Mimosa* included in this study may be considered a monophyletic taxon, sister to *Piptadenia viridiflora* (Fig. 2, BS = 80%). As discussed in the introduction, conflicts of delimitation were found between (a) sections *Batocaulon* and *Habbasia*, (b) sections *Calothamnos* and *Mimosa*, and (c) among series and subseries within section *Mimosa*, and are discussed below.

SECTIONS *BATOCAULON* AND *HABBASIA*

Section *Batocaulon* was defined as a highly polymorphic group (Barneby, 1991) mainly based on plain, cauline setae and setiform cilia on leaflet margins. However, these types of indumenta are also found in some representatives of sections *Habbasia* and *Mimosa*. The species analyzed here are grouped into two clusters; the first one includes *M. detinens* and *M. hexandra* (basal and highly supported), and the second includes *M. bifurca* and *M. uliginosa* (more recent and also highly supported). Our analysis suggests that species of section *Batocaulon* may have arisen from two speciation events; the first one gave the series *Farinosae* Benth. (*M. detinens*) and *Bimucronatae* Barneby (*M. hexandra*), and a later event gave rise to series *Stipellares* (*M. bifurca* and *M. uliginosa*). This result indicates that the section *Batocaulon* recognized by Barneby (1991) cannot be considered a natural group.

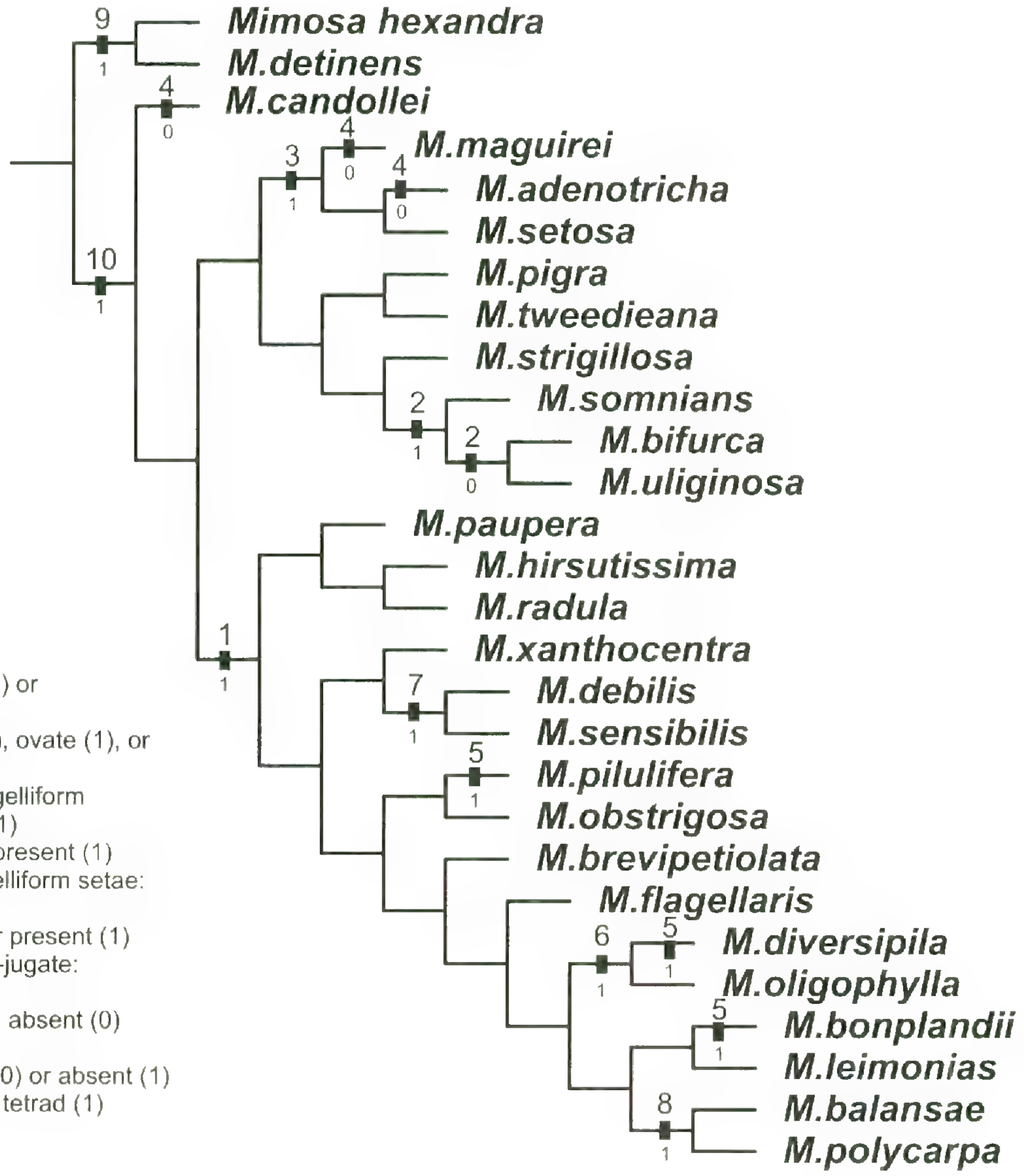
The position in the tree for *Mimosa detinens* and *M. hexandra* can be partially explained by the xerophilous habit and the bitetrad type pollen (Caccavari, 1985, 1986a, b, 1989; Martínez-Bernal et al., 2005) as shown by the optimization in the phylogeny hypothesis indicated in Figure 3 (characters 9 and 10). Moreover, the association between *M. detinens* and *M. hexandra* could also be explained by the flower's white filament (Burkart, 1948; Barneby, 1991). Series *Stipellares* is characterized by its particular foliar morphology having paraphyllidia at the base of pinna-rachis elliptic, reniform to suborbicular, simulating diminutive leaflets; no interpinnal spicules; and, in some species, pinnae and leaflets alternately inserted along the leaf axes. We mapped three types of paraphyllidia (Fig. 3, character 2) and determined that the foliaceous type could be considered a derived feature from the ovate and subulate types.

Within the clade in which all species of section *Habbasia* sensu Barneby are represented together along with section *Batocaulon* ser. *Stipellares*, two clades may be distinguished (Figs. 1, 2). The first includes the species of series *Pachycarpae* (*Mimosa maguirei*, *M. adenotricha*) and series *Setosae* (*M. setosa*). Barneby described the series *Setosae* in order to include the polymorphic *M. setosa* (1991: 352–353), which shows transitional traits between sections *Habbasia* and *Batocaulon*, but exhibits articulated pods with relatively narrow replum that differentiate it from those of series *Pachycarpae*. Although we include few representatives of series *Pachycarpae* and *Setosae*, the molecular results presented here suggest maintaining them as a single series. When the indumentum type typical of both series was mapped (Fig. 3, character 3), the bulbosus or flagelliform type appears at the base of the clade integrated by *M. maguirei*, *M. adenotricha*, and *M. setosa*; however, one might assume a parallel derivation of the valvate fruit type in *M. adenotricha*, *M. maguirei*, and *M. candollei* (Fig. 3, character 4).

In Figure 2, *Mimosa tweedieana* and *M. pigra* constituted a moderately supported clade (BS = 64%). Nevertheless, although it is in the same series, *M. strigillosa* is placed in another group. *Mimosa strigillosa* differs from other members of series *Habbasia* by its herbaceous life form, with stems humifuse, radicant; basally monadelphous stamens; and smaller, 2- to 5-seeded pods. Moreover, this species has a bicentric distribution (southeastern U.S.A. to northeastern Mexico and the basins of the Paraná and Paraguay rivers in Paraguay to northeastern Argentina and western Uruguay) that is unusual in the remaining species of series *Habbasia*. Although in the majority consensus tree (Fig. 1) it is not solved, in the selected phylogeny hypothesis (Fig. 2), *M. strigillosa* is closely related to *M. somnians* (*Bipinnatae*); despite this, the clade is weakly supported. The association between the latter species and the taxa included from section *Batocaulon* ser. *Stipellares* (*M. bifurca* and *M. uliginosa*) is also weak. However, this latter relation may be partially explained by the absence of interpinnal spicules and the plausible progression of paraphyllidia morphology (Fig. 3, character 2). Given that all the species of series *Bipinnatae* have striate nerved corollas not found in other members of *Habbasia* species, one might hypothesize that it is a natural group. Nevertheless, the tendency could not be tested in this study based on the limited number of taxa included.

SECTIONS *MIMOSA* AND *CALOTHAMNOS*

In our phylogenetic hypothesis, the species of sections *Mimosa* and *Calothamnos* are derived from a common ancestor (Figs. 1, 2). The position of the *Calothamnos* species can be explained by the presence of haplostemonous flowers (Fig. 3, character 1) as was proposed by Bentham (1841–1842, 1875). Although previous works (Grether et al., 2005) confirmed the monophyly of section *Calothamnos*, in our study, the two representatives of section *Calothamnos* analyzed are not clustered together. It may be that the section is underrepresented, but this analysis indicates that section *Calothamnos* is not monophyletic. The haplostemonous species with the indumentum plumose or consisting of stelliform setae were recognized by Bentham (1841) as section *Mimosa* ser. *Lepidotae* and transferred by Barneby (1991) to a new section *Calothamnos* (Table 1). However, when the indumentum plumose or stelliform setae trait is mapped onto the



Morphological characters

1- Flowers: diplostemonous (0) or haplostemonous (1)
2- Paraphyllidia: foliaceous (0), ovate (1), or subulate/setiform (2)
3- Indumentum bulbous or flagelliform based: absent (0) or present (1)
4- Craspedium: absent (0) or present (1)
5- Indumentum plumose or stelliform setae: absent (0) or present (1)
6- Virgate stems: absent (0) or present (1)
7- Leaf 1-jugate and leaflets 2-jugate: absent (0) or present (1)
8- Aculei at or below the node: absent (0) or present (1)
9- Xerophilous habit: present (0) or absent (1)
10- Pollen type: bitetrad (0) or tetrad (1)

Figure 3. Optimization of 10 morphological characters over the preferred topology. Changes are represented by boxes. Above branches, numbers indicate the characters according to the list, while the trait states are indicated below.

tree, a parallel derivation might also be considered (Fig. 3, character 5), indicating that these characters are of limited phylogenetic value.

Mimosa pilulifera (sect. *Calothamnos*) constitutes a clade with *M. obstrigosa* (ser. *Mimosa* subser. *Obstrigosae* (Benth.) Barneby), and *M. bonplandii* (sect. *Calothamnos*) is clustered with *M. leimonias* (Fig. 2, BS = 71%) (ser. *Mimosa* subser. *Pedunculosae* (Benth.) Barneby). The yielded groups are unexpected because these species exhibit different morphological characteristics, such as the type of indumentum (branched trichomes for *M. pilulifera* and *M. bonplandii*, retrorsely apressed setae for *M. obstrigosa*, and glabrous to setulose-ciliolate for *M. leimonias*), life form (shrub in *M. pilulifera*, *M. bonplandii*, and *M. obstrigosa* vs. herb in *M. leimonias*), division of leaves (pinnae 1-jugate in *M. leimonias*, *M. pilulifera*, and *M. obstrigosa* vs. pinnae 2- to 9-jugate in *M. bonplandii*), and androecium type and color of the filament (monadelphous and pale yellow in *M. bonplandii* vs. filaments free to the base and pink in the remaining species). The results suggest that these traits also exhibit low value from a phylogenetic standpoint.

As pointed out in the introduction, *Mimosa diversipila* shares the calyx and pubescence types with species of section *Calothamnos*, and has the virgate life form present in section *Mimosa* ser. *Mimosa* subser. *Brevipedes* (Barneby, 1991: 708–710). In cladograms, *M. diversipila* is clustered with *M. oligophylla* from section *Mimosa* ser. *Mimosa* subser. *Pedunculosae*. Although this association is weak, it can be partially expected because both show the simple virgate habit absent from the remaining species from subseries *Pedunculosae* analyzed here (Fig. 3, character 6).

SERIES AND SUBSERIES OF SECTION *MIMOSA*

Two well-supported groups are obtained: in the first, the species of subseries *Mimosa* (*M. debilis* and *M. sensibilis*) are clustered together in accordance with the Bentham (1875) (series *Sensitivae* Benth.) and Barneby (1991) subdivision based on the pinna 1-

Table 4. List of *Mimosa* species sequenced for this study. Taxa are organized according to Barneby's classification (1991).

Barneby (1991)	Species
Sect. *Habbasia* ser. *Bipinnatae*	*Mimosa somnians* Humb. & Bonpl. ex Willd.
Sect. *Habbasia* ser. *Habbasia*	*Mimosa strigillosa* Torr. & A. Gray
Sect. *Habbasia* ser. *Habbasia*	*Mimosa tweedieana* Barneby ex Glazier & Mackinder
Sect. *Habbasia* ser. *Habbasia*	*Mimosa pigra* L.
Sect. *Habbasia* ser. *Pachycarpae*	*Mimosa adenotricha* Benth.
Sect. *Habbasia* ser. *Pachycarpae*	*Mimosa maguirei* Barneby
Sect. *Habbasia* ser. *Setosae*	*Mimosa setosa* Benth. var. *setosa*
Sect. *Batocaulon* ser. *Stipellares*	*Mimosa uliginosa* Chodat & Hassl.
Sect. *Batocaulon* ser. *Stipellares*	*Mimosa bifurca* Benth.
Sect. *Batocaulon* ser. *Farinosae*	*Mimosa detinens* Benth.
Sect. *Batocaulon* ser. *Bimucronatae*	*Mimosa hexandra* Micheli
Sect. *Mimosa* ser. *Mimosa* subser. *Brevipedes*	*Mimosa diversipila* Micheli var. *diversipila*
Sect. *Mimosa* ser. *Mimosa* subser. *Hirsutae*	*Mimosa hirsutissima* Mart.
Sect. *Mimosa* ser. *Mimosa* subser. *Mimosa*	*Mimosa debilis* Humb. & Bonpl. ex Willd.
Sect. *Mimosa* ser. *Mimosa* subser. *Mimosa*	*Mimosa sensibilis* Griseb.
Sect. *Mimosa* ser. *Mimosa* subser. *Obstrigosae*	*Mimosa obstrigosa* Burkart
Sect. *Mimosa* ser. *Mimosa* subser. *Pedunculosae*	*Mimosa brevipetiolata* Burkart
Sect. *Mimosa* ser. *Mimosa* subser. *Pedunculosae*	*Mimosa flagellaris* Benth.
Sect. *Mimosa* ser. *Mimosa* subser. *Pedunculosae*	*Mimosa leimonias* Barneby & Fortunato
Sect. *Mimosa* ser. *Mimosa* subser. *Pedunculosae*	*Mimosa oligophylla* Micheli
Sect. *Mimosa* ser. *Mimosa* subser. *Polycarpae*	*Mimosa balansae* Micheli
Sect. *Mimosa* ser. *Mimosa* subser. *Polycarpae*	*Mimosa polycarpa* Kunth
Sect. *Mimosa* ser. *Mimosa* subser. *Polycephalae*	*Mimosa radula* Benth.
Sect. *Mimosa* ser. *Mimosa* subser. *Pudicae*	*Mimosa xanthocentra* Mart. var. *xanthocentra*
Sect. *Mimosa* ser. *Mimosa* subser. *Reptantes*	*Mimosa paupera* Benth.
Sect. *Calothamnos*	*Mimosa bonplandii* (Gillies ex Hook. & Arn.) Benth.
Sect. *Calothamnos*	*Mimosa pilulifera* Benth.
Sect. *Batocaulon* ser. *Quadrivalves*	*Mimosa candollei* R. Grether

jugate and leaflet 2-jugate present in all members of subseries *Mimosa* (Fig. 3, character 7). The second group is formed by *M. balansae* and *M. polycarpa*, both from subseries *Polycarpae* Barneby, which share some common traits, in particular the aculei at or below the nodes (Fig. 3, character 8). Because the species of subseries *Polycarpae* are at the tip in the molecular tree, it is suggested that they are the most recently differentiated group.

However, the position obtained for the species of section *Mimosa* does not agree completely with Barneby's classification. Examples of these discordances are the groupings of *M. hirsutissima* and *M. radula* that constitute a highly supported clade (Fig. 2, BS = 100%), although the first one belongs to subseries *Hirsutae* (Benth.) Barneby and the second to subseries *Polycephalae* (Fig. 2, Table 4). The species from subseries *Pedunculosae* (*M. flagellaris*, *M. oligophylla*, *M. brevipetiolata*, and *M. leimonias*) are not clustered, which shows that this subseries is an artificial group (Figs. 1, 2).

The low support of some clades confirms the idea that *Mimosa* is a recently diverged genus. Low divergence is expected in neutral markers, such as cpDNA introns and intergenic spacers that would not have had enough time to be fixed in different species and yield poorly supported nodes (Soltis & Soltis, 1998). Alternative explanations that cannot be ruled out are ancestral polymorphism in cpDNA or past introgression events. The latter, combined with strong directional selection in certain environments creating morphological divergence despite gene flow as postulated by Orr and Smith (1998), seems also to be a plausible explanation for *Mimosa*. In the center of diversity in south Amazonia in Brazil and adjacent areas in Paraguay, Argentina, and Uruguay, high hybridization among species from sections *Batocaulon* and *Mimosa* is described (Morales et al., 2007; Fortunato, unpublished data). The occurrence of natural hybrids followed by stabilization processes is necessary in speciation by hybridization (Grant, 1971), so the hybrids in the wild can be interpreted as evidence to suggest that interspecific hybridization plays an important role in the evolution of the genus *Mimosa*. Moreover, different ploidy levels were described in *Mimosa* in ca. 30 species (Withus & Berger, 1947; Goldblatt, 1981; Seijo, 1993, 1999, 2000; Morales et al., 2007). Morphological, cytogenetic, and molecular criteria indicated that hybridization and polypoloidy processes have been impor-

tant mechanisms in the evolution of other legumes (Hughes et al., 2002). However, a study on the relative importance of each process that could explain the evolution of *Mimosa* is still lacking.

Final Remarks

In this paper, we tested the monophyly of *Mimosa* and determined that it is a genus derived from *Piptadenia viridiflora*. Recently, Jobson and Luckow (2007) suggested that *P. viridiflora* should be removed from the genus *Piptadenia* based on chloroplast genes *trnL-trnF* and *trnK/matK*. Although representatives from section *Mimadenia* were not included in this study, representatives from sections *Batocaulon*, *Habbasia*, *Mimosa*, and *Calothamnos* constitute a single clade. Moreover, the sections analyzed here, accepted by Barneby (1991), are not monophyletic; in this regard, the only species that constitute a well-supported clade are those from sections *Mimosa* and *Calothamnos*. The most recent group exhibits the derived morphological features, as was proposed by Barneby (1991). The evolutionary sequence can be interpreted as a progressive reduction—from diplostemonous and free filaments to haplostemonous and an androecium with monadelphous filaments—in leaf formula, in habit life form (trees and shrubs to herb), and in pollen type (bitetrads to tetrads). However, in order to determine whether this molecular hypothesis is congruent with Barneby's proposal for relationships, a study adding representatives from *Mimadenia* is needed.

To conclude, ours is the first documented molecular study of the genus *Mimosa*. The cpDNA regions examined here provide sufficient information to resolve many relationships within the genus. This hypothesis, based on *trnL* and *trnL-trnF* intergenic spacer regions, constitutes a starting point for further analysis. It will be interesting to see whether more exhaustive sampling and additional genetic evidence (i.e., nuclear evidence or other cpDNA regions) will support the working hypothesis developed in this study.

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PHYLOGENETIC RELATIONSHIPS OF THE *LITSEA* COMPLEX AND CORE LAUREAE (LAURACEAE) USING ITS AND ETS SEQUENCES AND MORPHOLOGY[1]

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Abstract

Nuclear DNA ITS and ETS sequences of 71 representatives from nine genera and 11 sections of the core Laureae were combined with a matrix of morphological characters, analyzed using maximum parsimony with both equally and successively weighted characters, and analyzed for Bayesian inference, minimum evolution by neighbor joining, and maximum likelihood inference for molecular data alone. The large genera *Actinodaphne* Nees, *Lindera* Thunb., and *Litsea* Lam. were polyphyletic, as were *Lindera* sect. *Aperula* (Blume) Benth. and *Litsea* sections *Conodaphne* (Blume) Benth. & Hook. f., *Cylicodaphne* (Nees) Hook. f., and *Tomingodaphne* (Blume) Hook. f. In contrast, *Neolitsea* (Benth.) Merr. was monophyletic and terminal in a larger monophyletic lineage above an *Actinodaphne* grade. A major disparity exists between these molecular results and traditional morphology-based classifications within Lauraceae. These results suggest that the use of two- versus four-celled anthers for Laureae generic delimitation has resulted in polyphyletic or paraphyletic genera, and the character of dimerous versus trimerous flowers is of only limited phylogenetic value. Several of the major lineages in Laureae are supported by inflorescence morphology and ontogeny, with Laureae defined by short shoots with a vegetative terminal bud, splitting into thyrsoid (*Actinodaphne* and *Neolitsea*) versus racemose (*Laurus*, *Litsea* s. str., and *Lindera* s. str. and *Lindera* sect. *Aperula*), although there appear to be at least two different pathways to form the Laureae pseudo-umbel. Similarly, imbricate, early deciduous inflorescence basal involucral bracts defined an *Actinodaphne–Neolitsea Parasassafras–Sinosassafras* clade, although within it, *Neolitsea* was defined in part by decussate, persistent bracts. Accordingly, our study indicates the need for caution in the use of morphology for assessing affinity in Laureae, as virtually all traditional morphological characteristics show high levels of homoplasy and/or reversal, but future research may help to resolve whether this indicates problems of homology or ontogenetic convergence.

Key words: character evolution, inflorescence structure, Lauraceae, Laureae, phylogenetic classification.

The core Laureae comprise eight genera with approximately 500 species, mainly from tropical and subtropical Asia, making this region a center of generic and infrageneric diversification and distribution for the Laureae (Li, 1995). The group is defined morphologically by its dioecious breeding system, mostly pseudo-umbellate inflorescences subtended by involucral bracts, and introrse anther cells in the third whorl (Li & Christophel, 2000; Li et al., 2004). However, a large portion of what we now regard as the Laureae, including *Actinodaphne* Nees, *Dodecadenia* Nees, *Iteadaphne* Blume, *Laurus* L., *Lindera* Thunb., *Litsea* Lam., *Neolitsea* (Benth.) Merr., *Parasassafras* D. G. Long, and *Sinosassafras* H. W. Li, were only placed together relatively recently (Rohwer, 1993; van der Werff & Richter, 1996). Although half of the genera in the Laureae are monotypic or oligotypic, *Actinodaphne*, *Lindera*, *Litsea*, and *Neolitsea* each possess over 100 species.

Hooker (1890) and Li et al. (1984) divided *Litsea* into four sections, but these divisions were not explicitly phylogenetic. Section *Litsea* is evergreen with alternate, penninerved leaves, a racemiform inflorescence, and non-enlarged perianth tubes with

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reduced or absent perianth lobes. Section *Conodaphne* (Blume) Benth. & Hook. f. is evergreen with alternate or opposite penninerved leaves and non-enlarged or slightly enlarged perianth tubes, whereas section *Cylicodaphne* (Nees) Hook. f. represents evergreen taxa with alternate, penninerved leaves, an enlarged perianth tube, and cup-shaped fruiting cupule. In contrast, section *Tomingodaphne* (Blume) Hook. f. has deciduous, alternate, penninerved leaves and non-enlarged, six-lobed perianth tubes.

Tsui (1987) divided *Lindera* into eight sections, mainly following Hooker's (1890) criteria for *Litsea*, but also incorporating Li's (1985) concept of the "shortened brachyblast," which had been proposed to explain inflorescence evolutionary trends in Laureae. Section *Lindera* has taxa with deciduous, penninerved leaves and well-developed terminal buds on shortened brachyblasts; section *Sphaerocarpae* H. P. Tsui possesses deciduous, triplinerved or trinerved leaves and well-developed terminal buds on shortened brachyblasts; and section *Palminerviae* Meisn. was created for deciduous taxa with lobed, trinerved leaves and well-developed terminal buds on shortened brachyblasts. Section *Aperula* (Blume) Benth. has evergreen, penninerved leaves, well-developed terminal buds on shortened brachyblasts, and long-pedunculate, racemiform-arranged inflorescences; section *Cupuliformes* H. P. Tsui possesses evergreen, penninerved leaves, funnel-shaped glands on the third anther whorl, and enlarged perianth tubes forming cup-shaped fruiting cupules. Section *Daphnidium* (Nees) Benth. has evergreen, trinerved or triplinerved leaves, and non-developing terminal buds on shortened brachyblasts; section *Polyadenia* (Nees) Benth. is evergreen with penninerved leaves and ill-developed terminal buds on the axillary short shoots, whereas section *Uniumbellatae* H. P. Tsui has evergreen, trinerved leaves, and the long-pedunculate pseudo-umbel is solitary and borne in the axil of a normally developed leaf.

Generic delimitation in Laureae is problematic (Kostermans, 1957; Hutchinson, 1964; Richter, 1981), the major point being the significance of two- versus four-celled anthers as generic descriptors, although recent Lauraceae classifications tend to downplay this feature (e.g., Rohwer et al., 1991; Rohwer, 1993; van der Werff & Richter, 1996).

The most recent hypothesis about evolution within the Laureae was constructed under the assumption of maximum parsimony (MP) using nucleotide data from chloroplast DNA (cpDNA) *matK* and nuclear DNA (nrDNA) ITS (Li et al., 2004). This study included 22 species and one outgroup taxon, and shed light on major lineages, as well as on the relationships of some previously problematic genera. That study suggested that *Litsea* and *Lindera* are polyphyletic, with no support for characters such as two- versus four-celled anthers, and identified novel clades that appeared to reflect inflorescence structure and ontogeny. Nevertheless, although the molecular analyses provided some phylogenetic insights of the core Laureae, generic boundaries and relationships were still uncertain due to limited sampling and the fact that morphology was not included explicitly in the analyses. In addition, the level of *matK* divergence was particularly low in Laureae, resulting in poorly supported groupings.

To reconstruct a more reliable phylogeny of the core Laureae, it was necessary to sample additional taxa, as well as to utilize more informative DNA regions. The most widely used sources of molecular data for plant phylogenetic studies at the specific and generic levels are the ITS-1 and ITS-2 regions in nrDNA (Baldwin et al., 1995). However, for Lauraceae (Chanderbali et al., 2001) and Laureae (Li et al., 2004), these two spacers were too invariant or uninformative to provide robust node inferences by themselves. In contrast, although generally less easy to sequence than ITS, the nrDNA ETS has been shown to be useful for supplementing ITS data in plant phylogenetic studies (e.g., Baldwin & Markos, 1998; Clevinger & Panero, 2000; Markos & Baldwin, 2002; Plovanich & Panero, 2004), giving it the potential to provide additional characters in the Laureae.

In addition, because most recent molecular studies in Lauraceae have not explicitly included morphology as part of the analysis, preferring instead to discuss character patterns post hoc in relation to the molecular trees, there is a need for a combined analysis that includes both data sets in order to assess the impact, if any, of morphological data on the results.

Accordingly, we have expanded the ITS study of Li et al. (2004) to include 71 taxa from the Laureae, three outgroups, and partial sequences from the ETS region, as well as information from morphological characters considered to be useful or definitive above the species level within the Laureae. The main goals were to assess the robustness of previously defined clades that had shown weak bootstrap support, as well as to see if support for features such as inflorescence structure was maintained with more extensive sampling and followed the explicit inclusion of morphology into the analyses, rather than just post hoc by character mapping. In particular, the hypotheses being tested were (1) Laureae genera based on two- versus four-celled anthers are not phylogenetically supported; (2) *Litsea*, *Lindera*, and *Actinodaphne* are not monophyletic; (3) *Neolitsea* is monophyletic; and (4) inflorescence ontogeny is phylogenetically important in the Laureae.

Methods

Taxon sampling and DNA extraction

In addition to 21 samples used previously by Li et al. (2004), except for *Lindera tienchuanensis* W. P. Fang & H. S. Kung (sect. *Uniumbellatae*) and *L. thomsonii* C. K. Allen (sect. *Daphnidium*) for which there was no more available material, an additional 50 species were included to cover the sections within *Lindera* and *Litsea* variously recognized by Li et al. (1984), Bentham (1880), Hooker (1890), Kostermans (1957), and Tsui (1987), as well as to sample more extensively within *Neolitsea* (Table 1). Outgroup sampling included one species each from *Cinnamomum* Schaeff., *Sassafras* J. Presl, and *Umbellularia* (Nees) Nutt., as these genera were sister to the core Laureae in Chanderbali et al. (2001) and Li et al. (2004).

Total genomic DNA was isolated from silica gel–dried material or herbarium specimens following Doyle and Doyle (1987), as modified by Li et al. (2004). Because of suspected fungal contamination in the previous ITS sequences of *Sinosassafras flavinervia* (C. K. Allen) H. W. Li and *Neolitsea confertifolia* (Hemsl.) Merr. (Li et al., 2004), both were re-extracted.

Amplification, sequencing, and sequence alignment

The ITS and 5.8S regions were amplified following the general methodology of White et al. (1990) and polymerase chain reaction (PCR) primers of Chanderbali et al. (2001), using the minor modifications reported in Li et al. (2004). The complete ETS intergenic spacer (IGS) was amplified with the 18S-IGS and 26S-F primers following the protocols of Baldwin and Markos (1998) and Starr et al. (2003) using *Actinodaphne trichocarpa* C. K. Allen, *Lindera latifolia* Hook. f., *Lindera megaphylla* Hemsl., *Litsea cubeba* (Lour.) Pers., and *Neolitsea chuii* Merr. This procedure yielded a product between 2 and 3 kb, of which ca. 650 bases were sequenced using the 18S-IGS primer. A conserved region located about 450 bases from the ETS/18S boundary was selected to design a primer, ETS-1 (CCA GAA CTC GCA CTT GCT GAG CTT), and this region was amplified for all taxa using ETS-1 and 18S-IGS as primers. PCR amplification followed the short-distance PCR protocol of Baldwin and Markos (1998), with an annealing temperature of 55 C, and TaKaRa ExTaq (TaKaRa Biotechnology, Dalian, China). All PCR amplifications included negative controls to detect contamination.

Although most PCR amplifications resulted in a single band, some individuals produced two size classes of PCR product. To isolate each of the two bands, PCR products were purified and blunt-end ligated into the EcoRV sites of the pMD18-T Simple Vector (TaKaRa) using the Original TA Cloning Kit (available from TaKaRa), and the fragments obtained following the manufacturer's protocol. PCR products were purified using the QIAquick PCR purification Kit (Qiagen, Tokyo, Japan) following protocols provided by the manufacturer, and sequenced in both directions. Sequencing reactions used the same primers as amplification and were conducted on an Applied Biosystems (Foster City, California, U.S.A.) 3100 DNA automated sequencer.

Sequences were checked against GenBank for the taxa of closest matching sequences by means of the BLAST search. Sequence chromatogram output files were aligned initially and edited base by base with the program SeqMan II (Lasergene software package; DNASTAR Inc., Madison, Wisconsin, U.S.A.). The edited sequences were then realigned using ClustalX version 1.8 (Thompson et al., 1997), and additional manual adjustments were made, if necessary, using BioEdit (Hall, 1999). All sequences analyzed in this study were deposited in GenBank (Table 1).

Morphology

Morphological data were collected from personal observation of live plants, herbarium specimens, and the available literature. Nineteen morphological characters (Table 2) were chosen for character evolution and mapping because they have been regarded as important in delimiting genera within the *Litsea* complex, and/or between sections within *Lindera* or *Litsea* (Bentham, 1880; Hooker, 1890; Kostermans, 1957; Li et al., 1984; Li, 1985; Tsui, 1987; Rohwer, 1993; van der Werff & Richter, 1996; Li & Christophel, 2000; van der Werff, 2001; Li et al., 2004). A matrix of 19 discrete morphological characters was constructed for all of the taxa investigated (Table 3).

Phylogenetic analyses

We used several methods to reconstruct phylogenies and assess branch support for clades: MP with both equally and successively weighted characters, bootstrapping, Bayesian inference, minimum evolution (ME) by neighbor joining, and maximum likelihood (ML) inference. In this way, clades with a strong, consistent phylogenetic signal should be recovered regardless of the methodology.

MP analyses were performed using PAUP* version 4.01b10 (Swofford, 1999). Congruence of the ITS and ETS data sets was assessed using the partition homogeneity test (PHT; Farris et al., 1995) as

Table 1. List of samples, voucher collection information, and GenBank accession numbers.

Taxon	Voucher	Source	ETS	ITS
***Actinodaphne* Nees**				
A. cupularis (Hemsl.) Gamble	*Wuyishan Exped. 1693* (KUN 0601976)	Guizhou, China	AY817123	AY817113
A. forrestii (C. K. Allen) Kosterm.	*Li H.-W. 2* (HITBC)	Yunnan, China	AY934881	AY265399
A. henryi Gamble	*Li J. 2002032* (HITBC)	Yunnan, China	AY817130	AY817120
A. kweichowensis Yen C. Yang & P. H. Huang	*Li H.-Q. 40091* (KUN 0106643)	Guangxi, China	AY817124	AY817114
A. lecomtei C. K. Allen	*Li C.-Q. 3979* (IBK 00003410)	Guangxi, China	AY817122	AY817112
A. oborata (Nees) Blume	*Li H.-W. 1* (HITBC)	Yunnan, China	AY934880	AY265398
A. omeiensis (H. Liu) C. K. Allen	*Yang G.-H. 55824* (KUN 0047252)	Yunnan, China	AY817127	AY817117
A. paotingensis Yen C. Yang & P. H. Huang	*Hainan Exped. 962* (IBK 00003425)	Hannan, China	AY817128	AY817118
A. pilosa (Lour.) Merr.	*Xie L.-S. 613* (KUN 0047277)	Guangxi, China	AY817125	AY817115
A. trichocarpa C. K. Allen	*Sun B.-X. 0757* (KUN 0047286)	Yunnan, China	AY817126	AY817116
A. tsaii Hu	*Feng G.-M. 22638* (KUN 0047322)	Yunnan, China	AY817129	AY817119
***Dodecadenia* Nees**				
D. grandiflora Nees	*Wu C.-Y. et al. 75-1048* (KUN 0049206)	Tibet, China	DQ120552	AY265397
***Iteadaphne* Blume**				
I. caudata (Nees) H. W. Li	*Li H.-W. 27* (HITBC)	Yunnan, China	DQ120551	AY265396
***Laurus* L.**				
L. nobilis L.	*Li H.-W. 16* (HITBC)	Yunnan, China	DQ120553	AY265392
***Lindera* Thunb.**				
Sect. *Aperula* (Blume) Benth.				
L. latifolia Hook. f.	*Li J. 2002076* (HITBC)	Yunnan, China	DQ120541	DQ124264
L. longipedunculata C. K. Allen	*Li H. 11114* (KUN 0690203)	Yunnan, China	DQ120542	DQ124265
L. metcalfiana C. K. Allen	*Li H.-W. 8* (HITBC)	Yunnan, China	DQ120543	AY265408
Sect. *Cupuliformes* H. P. Tsui				
L. megaphylla Hemsl.	*Li H.-W. 7* (HITBC)	Yunnan, China	AY934882	AY265406
Sect. *Daphnidium* (Nees) Benth.				
L. chunii Merr.	*Li J. 2002134* (HITBC)	Guangxi, China	DQ120547	DQ124266
L. pulcherrima (Nees) Hook. f.	*Li J. 2002202* (HITBC)	Guangxi, China	DQ120548	DQ124267
L. villipes H. P. Tsui	*Li H. et al. 11876* (KUN 0762162)	Yunnan, China	DQ120550	DQ124268
Sect. *Lindera*				
L. kariensis W. W. Sm.	*Li H. et al. 15305* (KUN 0789698)	Yunnan, China	DQ120539	DQ124263
L. reflexa Hemsl.	*Nei M.-X. & Lai S.-K. 3768* (KUN 0100201)	Jiangxi, China	DQ120540	AY265407
Sect. *Palminerviae* Meisn.				
L. obtusiloba Blume	*Sino-Amer. Exped. 1308* (KUN 0151469)	Hubei, China	DQ120546	AY265411
Sect. *Polyadenia* (Nees) Benth.				
L. communis Hemsl.	*Li H.-W. 4* (HITBC)	Yunnan, China	DQ120544	AY265409
Sect. *Sphaerocarpae* H. P. Tsui				
L. fruticosa Hemsl. (= *L. neesiana* (Wall. ex Nees) Kurz)	*Li G. F. 63966* (KUN 0104915)	Sichuan, China	DQ120545	AY265410
***Litsea* Lam.**				
Sect. *Conodaphne* (Blume) Benth. & Hook. f.				
L. monopetala (Roxb.) Pers.	*Li J. 2002108* (HITBC)	Guangxi, China	DQ120527	DQ120602
L. umbellata (Lour.) Merr.	*Li H.-W. 24* (HITBC)	Yunnan, China	DQ120528	AY265404
L. variabilis Hemsl. var. *variabilis*	*Li J. 2002142* (HITBC)	Guangxi, China	DQ120529	DQ120603
L. variabilis var. *oblonga* Lecomte	*Li J. 2002115* (HITBC)	Guangxi, China	DQ120530	DQ120604
Sect. *Cylicodaphne* (Nees) Hook. f.				

Table 1. Continued.

Taxon	Voucher	Source	ETS	ITS
L. acutivena Hayata	*Li J. 2002199* (HITBC)	Guangxi, China	DQ120531	DQ120605
L. dilleniifolia P. Y. Pai & P. H. Huang	*Li H.-W. 19* (HITBC)	Yunnan, China	DQ120532	AY265405
L. elongata (Nees) Hook. f.	*Li J. 2002146* (HITBC)	Guangxi, China	DQ120533	DQ120606
L. garrettii Gamble	*Xia Y.-M. s.n.* (HITBC)	Yunnan, China	DQ120534	DQ120607
L. liyuyingi H. Liu	*Xia Y.-M. s.n.* (HITBC)	Yunnan, China	DQ120535	DQ120608
L. longistaminata (H. Liu) Kosterm.	*Xia Y.-M. s.n.* (HITBC)	Yunnan, China	DQ120536	DQ120609
L. panamanja (Nees) Hook. f.	*Li J. 2002028* (HITBC)	Yunnan, China	DQ120537	DQ120610
L. yaoshanensis Yen C. Yang & P. H. Huang	*Li J. 2002198* (HITBC)	Guangxi, China	DQ120538	DQ120611
Sect. *Litsea*				
L. glutinosa (Lour.) C. B. Rob.	*Li H.-W. 21* (HITBC)	Yunnan, China	AY934883	AY265403
Sect. *Tomingodaphne* (Blume) Hook. f.				
L. cubeba (Lour.) Pers.	*Li H.-W. 28* (HITBC)	Yunnan, China	DQ120523	AY265402
L. kingii Hook. f.	*Li J. 2002170* (HITBC)	Guangxi, China	DQ120524	DQ120599
L. rubescens Lecomte	*Li J. 2002094* (HITBC)	Yunnan, China	DQ120525	DQ120600
L. sericea (Wall. ex Nees) Hook. f.	*Li H. et al. 15299* (KUN 0789681)	Yunnan, China	DQ120526	DQ120601
***Neolitsea* (Benth.) Merr.**				
N. aurata (Hayata) Koidz. var. *aurata*	*Li J. 2002181* (HITBC)	Guangxi, China	DQ120557	DQ124270
N. aurata var. *chekiangensis* (Nakai) Yen C. Yang & P. H. Huang	*Zhang S.-Y. 5482* (KUN 0162041)	Zhejiang, China	DQ120558	DQ124271
N. brassii C. K. Allen	*Gray, B. 03911* (KUN 0793628)	Queensland, Australia	DQ120559	DQ124272
N. cambodiana Lecomte var. *glabra* C. K. Allen	*Li X.-G. 202474* (IBK 00009945)	Guangdong, China	DQ120560	DQ124273
N. chrysotricha H. W. Li	*Wu S.-G. 7095* (KUN 0106438)	Yunnan, China	DQ120561	DQ124274
N. chuii Merr.	*Li J. 2002063* (HITBC)	Yunnan, China	DQ120562	DQ124275
N. confertifolia (Hemsl.) Merr.	*Xi X.-Y. 414* (PE 1272040)	Hunan, China	DQ120563	DQ124276
N. dealbata (R. Br.) Merr.	*Gray, B. 03993* (KUN 0793630)	Queensland, Australia	DQ120564	DQ124277
N. homilantha C. K. Allen	*Li J. 2002071* (HITBC)	Yunnan, China	DQ120565	DQ124278
N. kwangsiensis H. Liu	*Wu S.-J. 3419* (IBK 00010186)	Hong Kong, China	DQ120566	DQ121279
N. levinei Merr.	*Li H.-W. 29* (HITBC)	Yunnan, China	AY934884	AY265401
N. lunglingensis H. W. Li	*Li J. 2002058* (HITBC)	Yunnan, China	DQ120567	DQ124280
N. ovatifolia Yen C. Yang & P. H. Huang var. *ovatifolia*	*Wu S.-J. 3246* (IBK 00010360)	Hong Kong, China	DQ120568	DQ124281
N. ovatifolia var. *puberula* Yen C. Yang & P. H. Huang	*Mao P.-Y. 03875* (KUN 0108307)	Yunnan, China	DQ120569	DQ124282
N. pallens (D. Don) Momiy. & H. Hara	*Qinhai-Tibet Exped. 5972* (KUN 0108358)	Tibet, China	DQ120570	DQ124283
N. phanerophlebia Merr.	*Deng L. 7511* (KUN 0108338)	Guangdong, China	DQ120571	DQ124284
N. pingbienensis Yen C. Yang & P. H. Huang	*Mao P.-Y. 04139* (KUN 0108220)	Yunnan, China	DQ120572	DQ124285
N. pinninervis Yen C. Yang & P. H. Huang	*Li J. 2002187* (HITBC)	Guangxi, China	DQ120573	DQ124286
N. polycarpa H. Liu	*Zhou Z.-K. et al. EXLS-0252* (KUN 0695675)	Yunnan, China	DQ120574	DQ124287
N. pulchella (Meisn.) Merr.	*Li J. 2002166* (HITBC)	Guangxi, China	DQ120575	DQ124288
N. sericea (Blume) Koidz.	*K. Midorikawa 2180* (KUN 0108215)	Honshu, Japan	DQ120576	DQ124289
Neolitsea sp.	*Li J. 2002070* (HITBC)	Yunnan, China	DQ120581	DQ124294
N. sutchuanensis Y. C. Yang var. *sutchuanensis*	*Zhao Z.-X. 64* (KUN 0108178)	Sichuan, China	DQ120577	DQ124290
N. sutchuanensis var. *gongshanensis* H. W. Li	*Feng G.-M. 6987* (KUN 0108134)	Yunnan, China	DQ120578	DQ124291
N. undulatifolia (H. Lév.) C. K. Allen	*Li J. 2002203* (HITBC)	Guangxi, China	DQ120579	DQ124292

Table 1. Continued.

Taxon	Voucher	Source	ETS	ITS
N. wushanica var. *pubens* Yen C. Yang & P. H. Huang	*Liu L.-H. 15149* (KUN 0162057)	Hunan, China	DQ120580	DQ124293
***Parasassafras* D. G. Long**				
P. confertiflora (Meisn.) D. G. Long	*Qian Y.-Y. 682* (KUN 0104558)	Yunnan, China	AY934885	AY265395
***Sinosassafras* H. W. Li**				
S. flavinervia (C. K. Allen) H. W. Li	*Liu Y.-H. s.n.* (HITBC)	Yunnan, China	AY934886	AY940451
Outgroup taxa				
Cinnamomum pittosporoides Hand.-Mazz.	*Li H. 5252* (KUN 0108156)	Yunnan, China	DQ120554	DQ124269
Sassafras tzumu (Hemsl.) Hemsl.	*Li H.-W. 15* (HITBC)	Yunnan, China	DQ120555	AY265391
Umbellularia californica (Hook. & Arn.) Nutt.	*van der Werff s.n.* (MO)	North America	DQ120556	AY265393

implemented in PAUP*, and because the PHT was non-significant, the data sets were combined for all subsequent analyses. Most parsimonious trees were obtained from 10,000 replicates of random taxon addition using equally weighted (EW) characters (Fitch, 1971) and tree bisection-reconnection (TBR) branch swapping (MULPARS off), followed by swapping on the shortest trees from this analysis with MULPARS on. These trees were then used to re-weight the characters according to the best fit of their rescaled consistency indices (Farris, 1989). New searches were performed with 1000 replicates using successive weighting (SW) until equilibrium was reached (Farris, 1969). Clade support was estimated using bootstrap resampling (Felsenstein, 1985), with 1000 replicates, TBR, EW, and MP optimality criteria performed on the combined weighted ITS and ETS data with resampling using all characters equally, regardless of weight.

The 19 morphological characters were analyzed using unweighted parsimony, with all characters treated as unordered. Tree search was performed with 1000 replicates of random taxon addition and TBR branch swapping (MULPARS on) in PAUP* version 4.01b10 (Swofford, 1999). The morphological data were then combined with the ITS and ETS data and analyzed using the same settings that were used in the morphological data set. Clade support was estimated with bootstrap resampling (Felsenstein, 1985) for 1000 replicates with TBR, EW, and MP optimality. Morphological character state changes were then plotted on one of the resulting most parsimonious combined analysis trees using MacClade (Maddison & Maddison, 2000).

Table 2. Morphological characters and character states traditionally considered to be taxonomically important at the generic and sectional level in Laureae.

1. Habit: evergreen (0), deciduous (1)
2. Leaf arrangement: alternate along stems (0), alternate and crowded at branchlet apices (1), verticillate or subverticillate (2)
3. Leaf venation: pinninerved (0), triplinerved (1), trinerved (2)
4. Inflorescence type: thyrsoid, without vegetative terminal bud in the main axis (0), short shoot (brachyblast) with vegetative terminal bud in the main axis (1)
5. Inflorescence: terminal or subterminal (0), axillary (1)
6. Inflorescence arrangement: panicle (0), raceme (1), fasciculate clustered (2)
7. Inflorescence: sessile (0), stipitate (1)
8. Flower number per inflorescence: >1(0), 1(1)
9. Involucres: absent (0), present (1)
10. Involucres: large (0), minute (1)
11. Involucres: imbricate (0), decussate (1)
12. Involucres: early deciduous (0), persistent (1)
13. Flower sex: bisexual (0), unisexual (1)
14. Basic floral number: dimerous (0), trimerous (1)
15. Perianth segment: present, perfect (0), imperfect, absent or early deciduous (1)
16. Anthers: two-locular (0), four-locular (1)
17. Pollen sacs of the third whorl: latrorse (0), introrse (1), extrorse (2)
18. Fruit shape: globular or oblate (0), ovoid or ellipsoid (1)
19. Fruit cupule shape: flat or discoid (0), cup-shaped (1)

Bayesian phylogeny reconstruction of the combined data was performed with MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001; Huelsenbeck et al., 2002). The program Modeltest (Posada & Crandall, 1998) was used to test which of the 56 predicted models of DNA substitution best fit the data. Modeltest indicated that the TrN + I + Γ model with rate heterogeneity and among-site rate variation from Tamura and Nei (1993) best fit the two nrDNA regions, and the ML parameters in MrBayes (MB) were "lst nst = 6" and "rates = invgamma." The Markov chain Monte Carlo process was set so that four chains ran simultaneously for 500,000 generations, with trees sampled every 100 generations, giving a total of 5000 trees in the initial sample. Likelihood value plots for the four chains showed that stationarity had occurred by the 600th tree. Therefore, the first 600 trees were discarded as "burn in," and the posterior probabilities of the phylogeny and its branches were determined from the remaining 4400 trees.

ME analysis was also performed using neighbor joining on the unweighted data set using MEGA 3.1 (Kumar et al., 2004), with bootstrap support calculated on 10,000 replicates.

ML analysis of the combined, unweighted data was performed using the DNAML option in DAMBE version 4.13 (Xia, 2000; Xia & Xie, 2001).

Results

Sequence characteristics

ITS regions are quite variable between distantly related taxa, so only regions that could be aligned unequivocally were used in this analysis, making our phylogenetic estimates conservative. For the ingroup taxa, the length of the ITS regions, including the 5.8S region, ranged from 568 to 627 bp, and the alignment generated a data set of 689 characters of which 156 (22.64%) were parsimony informative. G plus C content ranged from 64.61% to 74.19%.

Compared to ITS, the approximately 400 bp of the 3′ end of the ETS between the 18S subunit and the ETS-1 internal primer were relatively easy to amplify and sequence. For the ingroup taxa, the ETS region varied in length from 350 to 393 bp, and alignment resulted in a matrix of 393 characters of which 99 (25.19%) were parsimony informative, with G plus C content ranging from 46.56% to 55.23%. All sequences were submitted to GenBank (Table 1), and the data matrices for both sequenced regions are available from the primary author upon request.

Phylogenetic analysis

SW parsimony. Results of phylogenetic analyses of the ITS and ETS regions performed separately showed no hard incongruences (i.e., there were no contradictory clades supported by bootstrap greater than 60%; data not shown). The PHT (Farris et al., 1995) indicated that the two regions were congruent ($P < 0.01$; see discussions in Sullivan, 1996; Cunningham, 1997; Farris et al., 2000). Because of this and because the ETS and ITS regions occur within the same transcriptional unit and show evidence of a similar and interdependent role in the maturation of ribosomal RNAs (Good et al., 1997), we will only present results for the combined analyses.

The EW analysis produced 30,600 trees (length [L] = 1171, consistency index [CI] = 0.306, retention index [RI] = 0.589), but because all main and virtually all minor branches collapsed under strict consensus, SW was used to try to stabilize the tree topology. SW reduced this to 12 trees (L = 211.5, CI = 0.576, RI = 0.834), producing an almost fully resolved strict consensus tree (Fig. 1).

The SW analysis of the nrDNA data produced a series of clades within a monophyletic core Laureae (93% bootstrap support). These clades are referred to informally as the *Neolitsea–Actinodaphne*, and *Litsea*, *Lindera*, and *Aperula* clades. These sat above a basal grade of *Lindera obtusiloba* Blume (sect. *Palminerviae*) and then *Lindera communis* Hemsl. (sect. *Polyadenia*). The *Aperula* clade contained the three sampled species of *Lindera* sect. *Aperula* (*L. latifolia*, *L. longipedunculata* C. K. Allen, and *L. metcalfiana* C. K. Allen) grouped in a terminal pairing with *Litsea cubeba* and *L. kingii* Hook. f. (both section *Tomingodaphne*) above a subclade of *Lindera megaphylla* (sect. *Cupuliformes*) and *Actinodaphne forrestii* (C. K. Allen) Kosterm. Although the *Aperula* clade as a whole was unsupported (< 50% bootstrap), all the branches within it had moderate (> 70%) to strong support (> 90%).

Sitting above *Sinosassafras* and *Parasassafras* was the *Lindera* clade (75% bootstrap support), with two subclades. The first of these represented *Lindera* sect. *Lindera* (2 spp.), plus *Litsea* species from sections *Tomingodaphne* and *Conodaphne* (part). Sister to this was a subclade consisting of *L. fruticosa* Hemsl. (sect. *Sphaerocarpae*), *Iteadaphne caudata* (Nees) H. W. Li, and the three species of *Lindera* sect. *Daphnidium*, again with most terminal branches moderately supported.

The *Litsea* clade consisted of a terminal lineage representing members of sections *Litsea*, *Conodaphne* (part), and *Cylicodaphne*, but also including *Dodecadenia grandiflora* Nees, all sitting above *Laurus nobilis* L. and *Actinodaphne lecomtei* C. K. Allen. The clade included within it three separate lineages. Section *Litsea* and *Litsea monopetala* (Roxb.) Pers. (sect. *Conodaphne*) formed a strongly supported pair

(99%), sister to a subclade to three species of *Litsea* sect. *Cylicodaphne*: *L. dilleniifolia* P. Y. Pai & P. H. Huang, *L. garrettii* Gamble, and *L. panamanja* (Nees) Hook. f. (here called *Cylicodaphne* I), and then to an *L. variabilis* Hemsl. var. *variabilis* and var. *oblonga* Lecomte (sect. *Conodaphne*) subclade, all with moderate to strong bootstrap support. The other branch in the *Litsea* clade was the *Cylicodaphne* II subclade, representing the remainder of *Litsea* sect. *Cylicodaphne* but including an embedded *Dodecadenia grandiflora*, again with most branches showing at least moderate support.

Within the *Neolitsea–Actinodaphne* clade, *Actinodaphne* (except *A. forrestii* and *A. lecomtei*) formed a basal grade to a well-supported *Neolitsea* (89%), with the latter divided into two subclades (*Neolitsea* I and *Neolitsea* II) above *N. chrysotricha* H. W. Li and *N. pallens* (D. Don) Momiy. & H. Hara. *Neolitsea* I showed little clear support for the internal branches, whereas all the branches in *Neolitsea* II showed bootstrap support > 50%.

Bayesian analysis. Bayesian analysis of the unweighted Laureae ITS + ETS showed moderate relationship resolution (Fig. 2), with the Bayesian tree corresponding well with much of the SW tree in terms of recovered major lineages. Although not as well resolved as the SW topology, terminal SW clades with high bootstrap support were also present in the Bayesian topology with strong posterior probability support, and both analyses included the *Neolitsea–Actinodaphne* clade and many of the major SW subclades.

Nevertheless, there were differences between the results for the two approaches. In the Bayesian tree, the *Lindera* clade was not recovered, with section *Daphnidium* falling instead as part of a polytomy separate from the remainder. Similarly, *Litsea glutinosa* (Lour.) C. B. Rob. (sect. *Litsea* and the type species for the genus) and *L. monopetala* (sect. *Conodaphne*) were separated from the rest of the *Litsea* clade seen in the SW analysis.

ME and ML analyses. The ME (neighbor joining) tree (Fig. 3) was well resolved and similar in major clade structure to the SW and Bayesian cladograms. *Neolitsea* I and II (albeit the latter reduced) were again terminal above an *Actinodaphne* grade, although here *A. paotingensis* Yen C. Yang & P. H. Huang was embedded in a basal *Neolitsea* subclade with *N. brassii* C. K. Allen, *N. wushanica* var. *pubens* Yen C. Yang & P. H. Huang, and *N. sutchuanensis* Y. C. Yang. *Laurus* was basal to the *Actinodaphne–Neolitsea* clade, and sister to this group was a clade consisting of part of the MP and MB *Aperula* clade and an expanded *Litsea* clade including the *Litsea* and *Cylicodaphne* I and II subclades, as well as *Dodecadenia* and *Iteadaphne*. Below this was a lineage containing the *Lindera*, *L. fruticosa*, and *Daphnidium* subclades, and then a polytomy consisting of *L. communis* paired with *Sinosassafras*, and an "*Aperula* II" clade of *Tomingodaphne*, *Cupiliformes*, and *Actinodaphne forrestii*. *Lindera obtusiloba* and then *Parasassafras* were basal to the remainder of Laureae.

Unlike most of the other analyses, in the ML tree (Fig. 4), *Lindera obtusiloba* and *L. communis* were paired and sister to the *Aperula* clade, with this lineage placed above *Laurus* and below the *Actinodaphne–Neolitsea* clade. *Neolitsea* I and II and the *Actinodaphne* grade were all present, although *A. paotingensis* was placed inside *Neolitsea* I, similar to the ME tree, and a subclade of *N. aurata* (Hayata) Koidz. and *N. kwangsiensis* H. Liu was basal to *Neolitsea* as a whole. Below all of these was a major lineage consisting of a *Lindera* clade, *L. fruticosa*, and the *Daphnidium* clade (with *Iteadaphne* basal) and a *Litsea* clade (including *Litsea* and the *Cylicodaphne* I and II subclades). *Actinodaphne lecomtei* was placed well inside the *Actinodaphne* grade, whereas *A. forrestii* was still sister to *Lindera megaphylla*, but this latter pair was placed between *Sinosassafras* and *Parasassafras* at the base of the Laureae.

Combined molecular and morphological analyses. Analysis of the morphological matrix by itself resulted in 317 equally parsimonious trees (L = 72, CI = 0.3056, RI = 0.8214), but these collapsed completely to an unresolved polytomy under strict consensus. When the morphological and molecular data were combined, two trees of 1271 steps (CI = 0.3021, RI = 0.6086) resulted, one of which is shown in Figure 5. This tree also recovered the *Neolitsea–Actinodaphne* clade, *Lindera* clade, and *Litsea* clade, which were seen in the molecular analysis, but the *Aperula* clade was now split into two separate entities, with *Aperula* I representing *Lindera* sect. *Aperula* s. str. and *Aperula* II representing *Litsea cubeba* and *L. kingii* (sect. *Tomingodaphne*), *Lindera megaphylla* (sect. *Cupuliformes*), and *Actinodaphne forrestii*. The inclusion of the morphological data changed the bootstrap support for most clades, generally lowering it from the SW analysis, although there was still reasonable bootstrap support for the terminal branches and still little or no deep-branch support.

When the morphological character state changes are plotted on the combined analysis tree (Fig. 5), the unique synapomorphy for the Laureae is the presence of inflorescences with short shoots (brachyblasts) with a vegetative terminal bud on the main axis. Within the Laureae, the *Actinodaphne–Neolitsea–Parasassafras–*

Table 3. Data matrix of important morphological characters in Laureae for species used in the molecular analyses.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Actinodaphne cupularis	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
Actinodaphne forrestii	0	2	0	1	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
Actinodaphne henryi	0	2	0	0	1	1	1	0	1	0	0	0	1	1	0	1	1	0	1
Actinodaphne kweichowensis	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	0	1
Actinodaphne lecomtei	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
Actinodaphne obovata	0	2	1	0	1	1	1	0	1	0	0	0	1	1	0	1	1	1	0
Actinodaphne omeiensis	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	0	0
Actinodaphne paotingensis	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	?	1
Actinodaphne pilosa	0	2	0	0	1	0	1	0	1	0	0	0	1	1	0	1	1	0	0
Actinodaphne trichocarpa	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	0	0
Actinodaphne tsaii	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
Cinnamomum pittosporoides	0	0	1	0	0	0	1	0	0	—	—	—	0	1	0	1	2	0	1
Dodecadenia grandiflora	0	0	0	1	1	2	0	1	1	0	1	1	1	1	0	1	0	1	0
Iteadaphne caudata	0	0	1	1	1	1	0	1	1	0	1	1	1	1	0	0	0	0	0
Laurus nobilis	0	0	0	1	1	1	1	0	1	0	1	1	1	0	0	0	1	1	0
Lindera chunii	0	0	2	1	1	2	1	0	1	0	1	1	1	1	0	0	1	1	0
Lindera communis	0	0	0	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
Lindera fruticosa	1	0	1	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
Lindera kariensis	1	0	0	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
Lindera latifolia	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
Lindera longipedunculata	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
Lindera megaphylla	0	1	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	1	1
Lindera metcalfiana	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
Lindera obtusiloba	1	0	2	1	1	2	1	0	1	0	1	1	1	1	0	0	1	1	0
Lindera pulcherrima	0	0	2	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
Lindera reflexa	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	0	1	0	0
Lindera villipes	0	0	2	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
Litsea acutivena	0	1	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	1	1
Litsea cubeba	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
Litsea dilleniifolia	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	0	1
Litsea elongata	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	1	1
Litsea garrettii	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1
Litsea glutinosa	0	0	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	0
Litsea kingii	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
Litsea liyuyingi	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	?	?
Litsea longistaminata	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0
Litsea monopetala	0	0	0	1	1	2	1	0	1	0	1	1	1	1	1	1	1	1	0
Litsea panamanja	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1
Litsea rubescens	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
Litsea sericea	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
Litsea umbellata	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	1
Litsea variabilis var. *variabilis*	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
Litsea variabilis var. *oblonga*	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
Litsea yaoshanensis	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	1	1
Neolitsea aurata var. *aurata*	0	1	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea aurata var. *chekiangensis*	0	1	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea brassii	0	2	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea cambodiana var. *glabra*	0	2	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea chrysotricha	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea chuii	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea confertifolia	0	2	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea dealbata	0	2	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea homilantha	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea kwangsiensis	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea levinei	0	2	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea lunglingensis	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0

Table 3. Continued.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Neolitsea ovatifolia var. *ovatifolia*	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea ovatifolia var. *puberula*	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea pallens	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea phanerophlebia	0	2	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea pingbienensis	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea pinninervis	0	1	0	0	1	2	1	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea polycarpa	0	1	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea pulchella	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea sericea	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Neolitsea sp.	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea sutchuanensis var. *sutchuanensis*	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea sutchuanensis var. *gongshanensis*	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
Neolitsea undulatifolia	0	2	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	1
Neolitsea wushanica var. *pubens*	0	0	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
Parasassafras confertiflora	0	0	1	1	1	1	1	0	1	1	0	0	1	1	0	1	0	0	0
Sassafras tzumu	1	1	0	0	0	1	1	0	1	0	0	1	1	1	0	1	1	0	0
Sinosassafras flavinervia	0	0	1	1	1	2	1	0	1	1	0	0	1	1	0	0	0	0	0
Umbellularia californica	0	0	0	0	1	1	1	0	1	0	0	0	0	1	0	1	2	1	0

Sinosassafras clade is united by the presence of imbricate, early deciduous involucral bracts, although *Neolitsea* itself has decussate persistent bracts as one of its synapomorphies. *Parasassafras* and *Sinosassafras* are supported as a pair by the possession of triplinerved leaves, minute involucral bracts, and latrorse pollen sacs for the third whorl, whereas the monophyly of the *Neolitsea–Actinodaphne* clade is supported by the synapomorphies of verticillate or subverticillate leaves and thyrsoid inflorescences that lack a terminal bud. Although there are few characters supporting the main branches within the *Actinodaphne* grade, *Neolitsea* is well supported, with triplinerved, alternate, crowded leaves at branch apices, decussate persistent bracts, and dimerous flowers. Within *Neolitsea*, there were no morphological synapomorphies supporting clades I and II, but within I, the species above *N. ovatifolia* Yen C. Yang & P. H. Huang var. *ovatifolia* (Fig. 5Ia) have verticillate or subverticillate leaves as a synapomorphy (albeit with some reversals), whereas in clade II there are two lineages: IIa, with ovoid to ellipsoid fruits, and IIb, defined by alternate leaves (again with a reversal in *N. brassii*).

In contrast, there were relatively no morphological synapomorphies supporting the *Lindera* or *Aperula* I or II clades, although the *Laurus–Litsea* clade had racemose inflorescences, and the *Litsea* clade was defined by four-locular anthers and the *L. glutinosa–L. monopetala* pair by the possession of imperfect, absent or early deciduous perianth segments. Within the *Litsea* clade, the *Cylicodaphne* II subclade was characterized by cup-shaped cupules and united with *Dodecadenia* on fasciculate, clustered inflorescences and ovoid/ellipsoid fruits. Similarly, within the *Lindera* clade, section *Daphnidium* was united by trinerved leaves and ovoid-ellipsoid fruits.

Discussion

Phylogenetic Utility of ETS in the Laureae

Several studies have shown that greater resolution and support for phylogenetic estimation are achieved by increasing character number and/or taxon representation (Graybeal, 1998; Hillis, 1998; Soltis et al., 1998; Bremer et al., 1999), and our study supports the importance of adding data from both more taxa and more sequence regions to help resolve issues in Laureae. The usefulness of the ETS region in molecular systematics has been suggested previously (Baldwin & Markos, 1998; Bena et al., 1998; Clevinger & Panero, 2000; Linder et al., 2000), and although the sequenced ETS segment is much shorter than the segment in the ITS (393 bp vs. 689 aligned bp) in Laureae, it nevertheless produces a slightly higher percentage of informative sites (25.19%) than that in the ITS (22.64%). The PHT for the two data sets showed congruence, and the combined analysis of the ITS and ETS sequences provided greater resolution and increased support for the relationships than either sequence by itself (trees not shown). This is consistent with the results of other combined ITS/ETS investigations (e.g., Li et al., 2002; Becerra, 2003; Lee et al., 2003; Morgan, 2003; Saar et al., 2003; Urbatsch

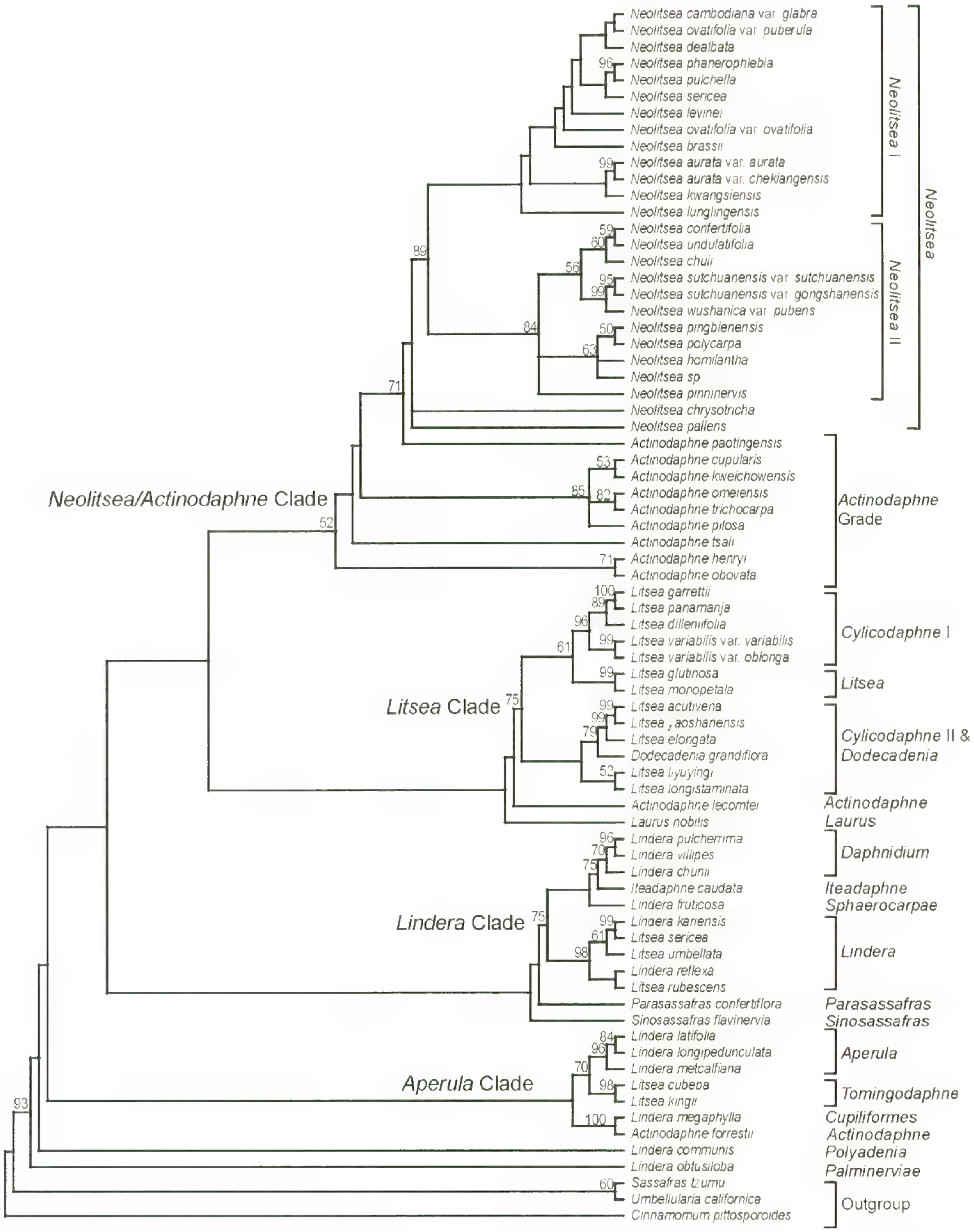

Figure 1. Strict consensus from 12 trees resulting for Laureae after successive weighting of the combined ITS and ETS sequence data. Bootstrap values greater than 50% are indicated on branches.

et al., 2003; Plovanich & Panero, 2004; Roalson & Friar, 2004).

RELATIONSHIPS AMONG MAJOR CLADES

Laureae are a well-supported monophyletic group based on our molecular data, and they share a morphological synapomorphy of pseudo-umbellate, rarely racemose or paniculate involucrate inflorescences. The results of our analyses are, in general, similar to the ITS + *matK* phylogeny of Li et al. (2004), and the four clades of Laureae and other subgroups recognized in that study more or less correspond to clades recovered here. Increased taxon

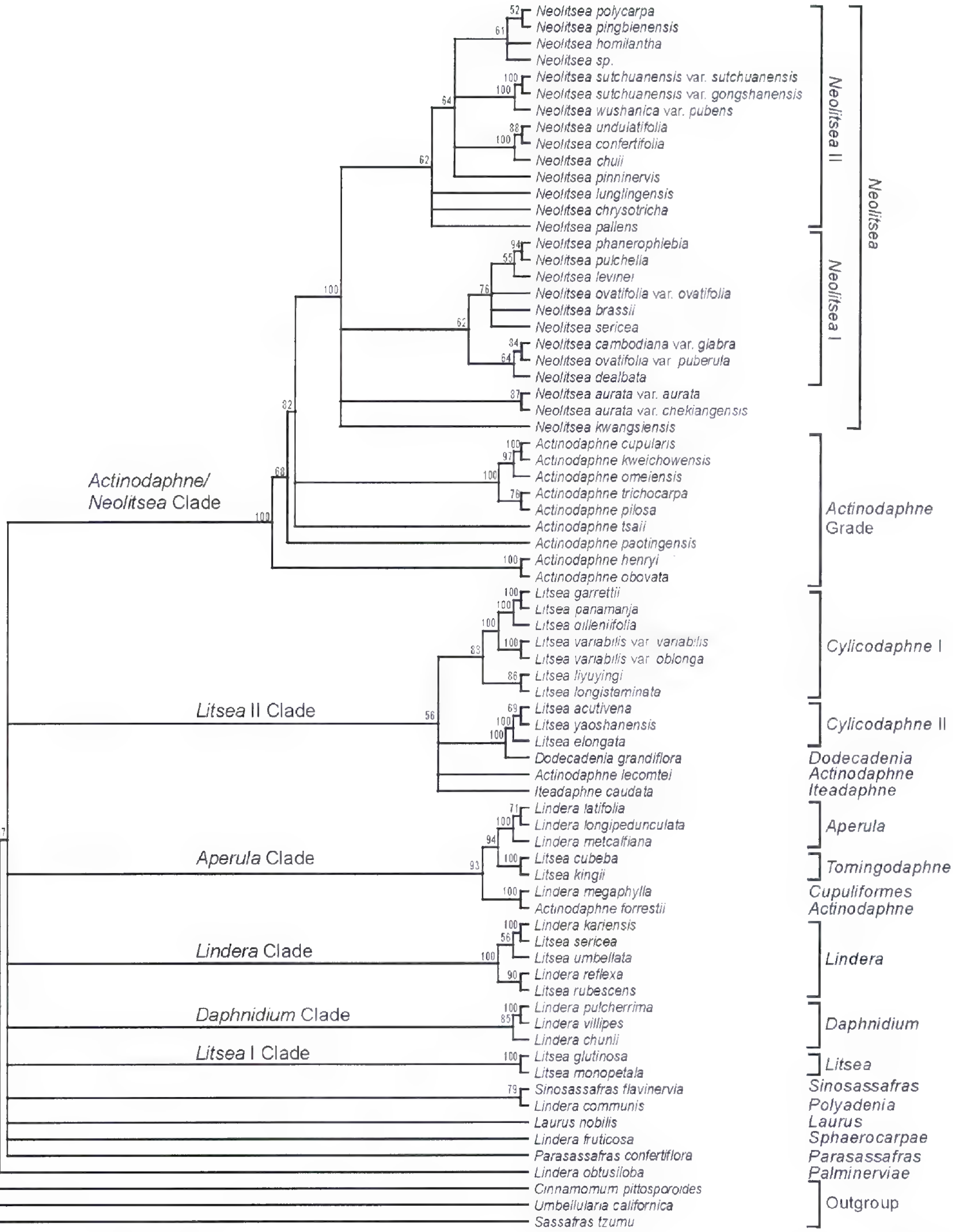

Figure 2. Phylogenetic majority rule tree for Laureae from Bayesian inference analysis of the unweighted combined ITS and ETS sequence data showing posterior probabilities on branches.

sampling as well as the additional sequence data from the ETS region and morphology have largely improved bootstrap, but mainly in more derived regions, with deep branch relationships still unresolved. This suggests that although main lineages are being identified and supported, the positions of many of these clades are unstable under different analysis procedures (e.g., *Lindera obtusiloba* basal to the Laureae; the isolated, generally basal position of *L. communis*; and the clades *Lindera* and *Aperula* less derived than the *Litsea*, *Laurus*, and *Actinodaphne–Neolitsea* clades). The precise relationships between major clades are, thus, still uncertain, suggesting that the sequence regions are evolving too rapidly to provide

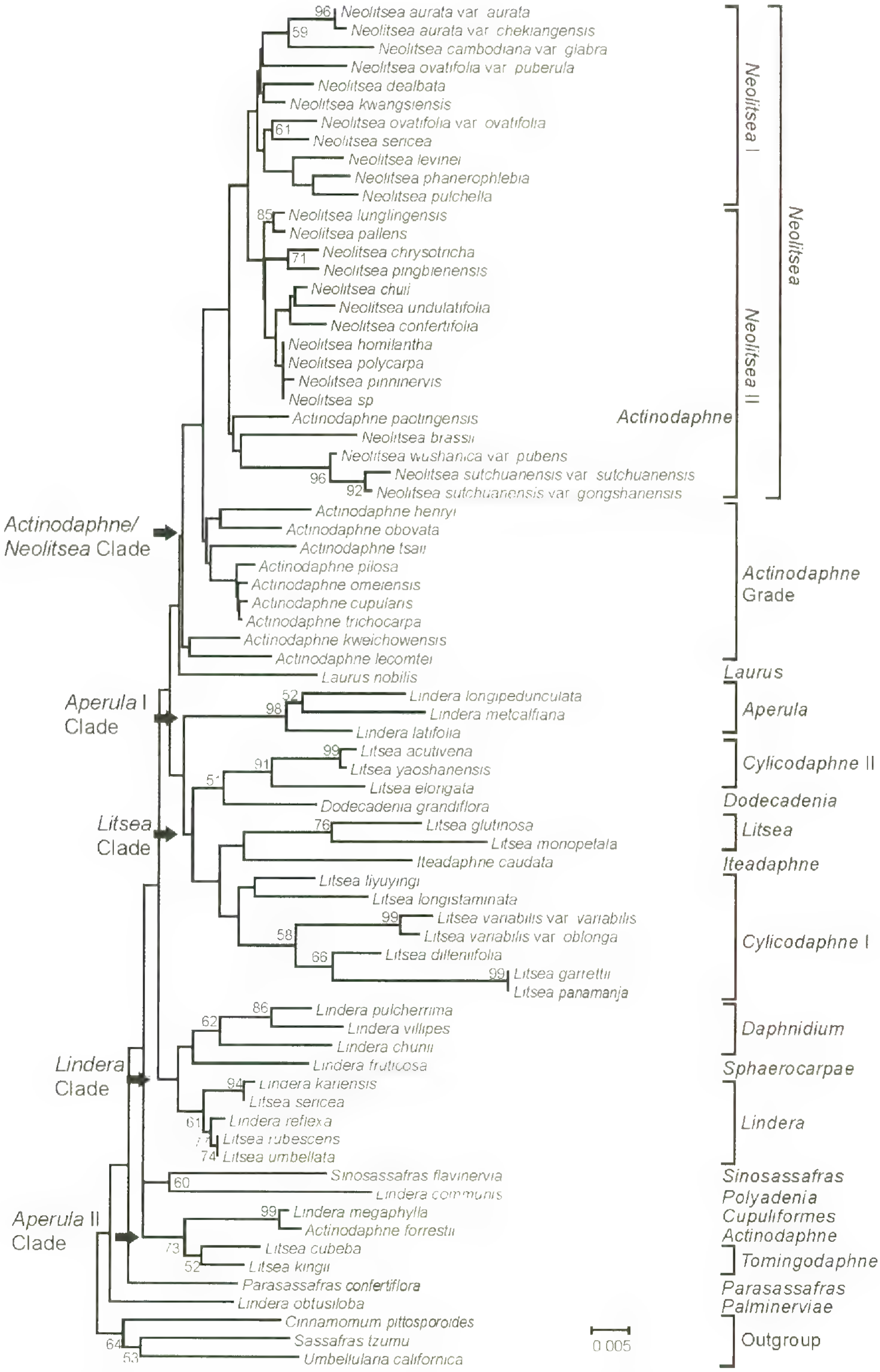

Figure 3. Minimum evolution (neighbor joining) tree for Laureae of the unweighted combined ITS and ETS sequence data. Bootstrap values (10,000 replicates) receiving support greater than 50% are indicated on branches.

deep-branch resolution, and sequences from slower-evolving gene regions may be needed to stabilize and support relationships between the major clades.

However, despite this caveat, our study includes representative taxa from all genera and most sections of Laureae, and the resulting clades support the hypothesis of Li et al. (2004) that inflorescence features and ontogeny are important for helping to understand evolution and improve classification within the Laureae.

The moderately supported association between *Sinosassafras flavinervia* and *Lindera communis* (sect. *Polyadenia*) in the MB and ME analyses also suggests

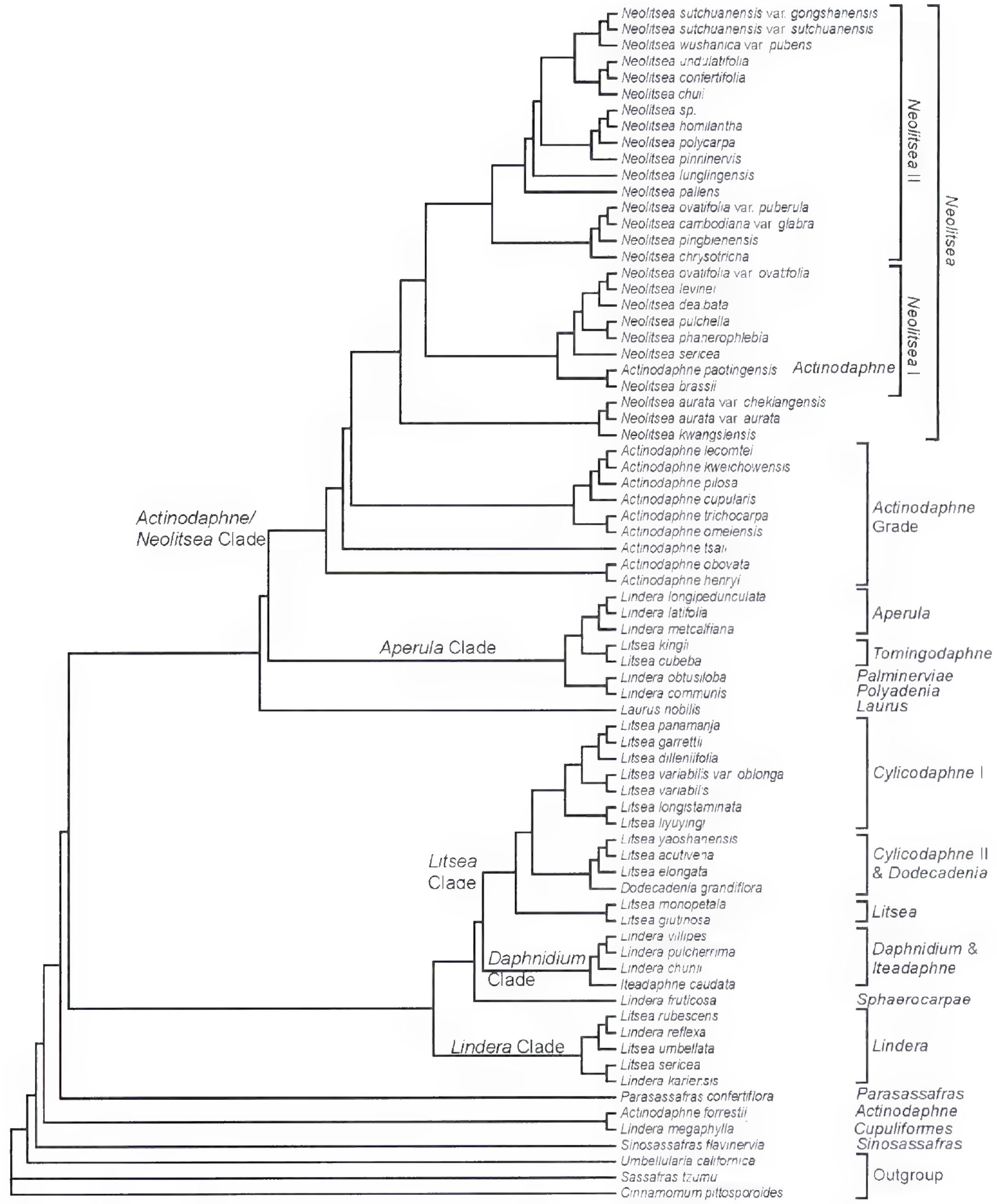

Figure 4. Maximum likelihood tree for Laureae of the unweighted combined ITS and ETS sequence data.

that the former may not be as closely related to *Parasassafras* as previously suggested (Rohwer, 1993), although these two genera were placed successively as basal to the *Lindera* clade in the SW analysis (albeit without bootstrap support) and formed a clade in the combined molecular/morphology analysis, with three morphological synapomorphies. *Sinosassafras flavinervia* has a single or two(to three) pseudo-umbels clustered in the leaf axils and always bears several pseudo-umbels on axillary short shoots, whereas *L. communis* only has a single or rarely two(to three) pseudo-umbels clustered in leaf axils. Nevertheless, in both taxa, the terminal buds on the axillary short shoots are poorly developed or reduced.

The *Aperula* II clade contained two subgroups (none of which actually belong to *Lindera* sect. *Aperula* s. str.): (1) *Litsea cubeba* and *L. kingii*; and (2) *Lindera megaphylla* and *Actinodaphne forrestii*, and three of

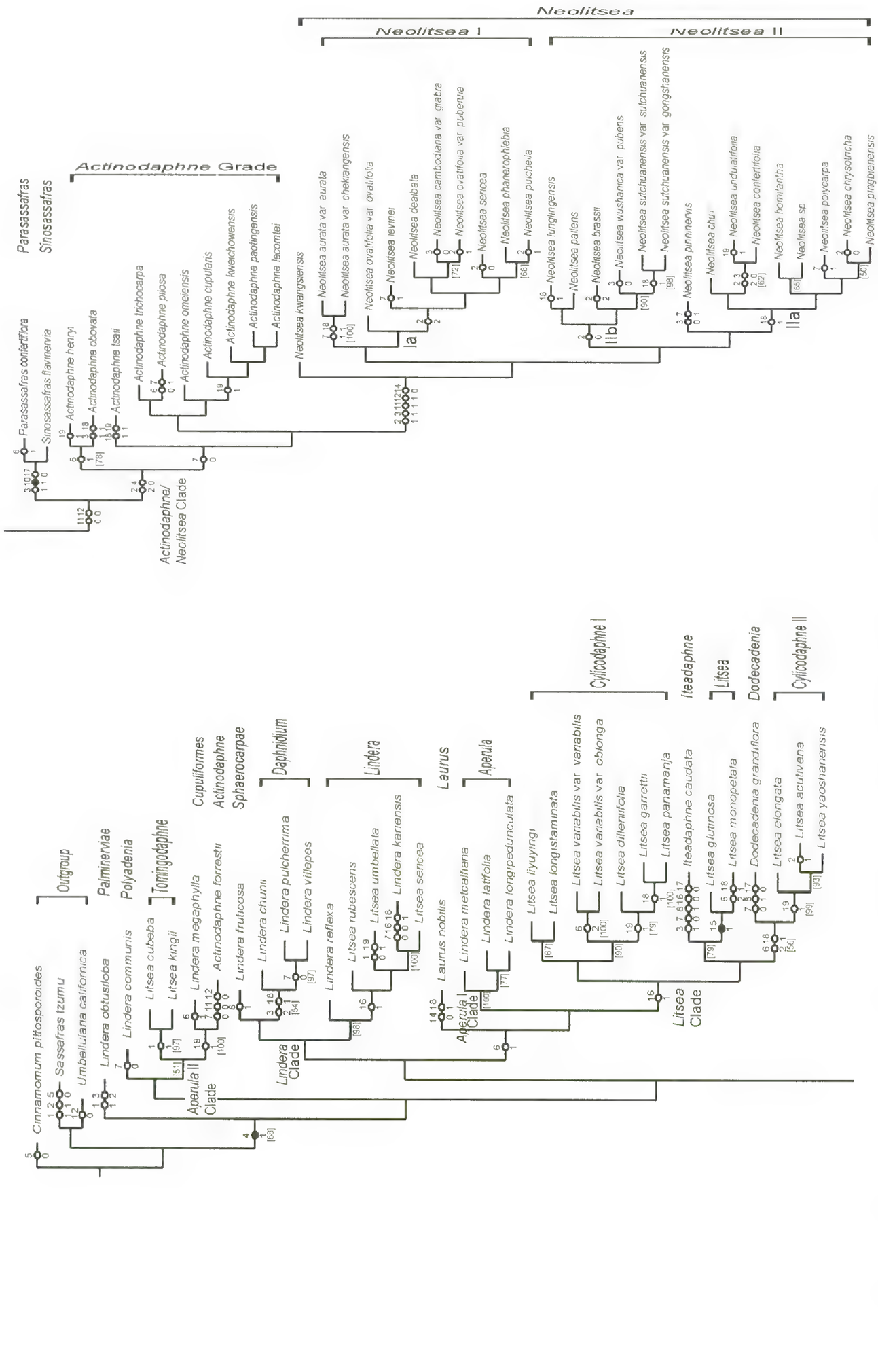

Figure 5. One of the two trees resulting for Lauraceae from unweighted combined ITS, ETS, and morphological data sets. Morphological character state changes are also plotted. Numbers above branches are characters; those below are character states from Table 2. Filled circles are unique state changes; open circles represent homoplasious changes. Bootstrap values greater than 50% are indicated in brackets below branches.

these (*Litsea cubeba*, *Lindera megaphylla*, and *A. forrestii*) also formed a clade in the study by Li et al. (2004). *Litsea cubeba* and *L. kingii* (sect. *Tomingodaphne*) are united by being deciduous, with naked terminal buds, and differ mainly in the absence of leaf pubescence in *L. kingii* (Long, 1984). Li et al. (2004) also noted that there was a close relationship between *Lindera megaphylla* (sect. *Cupuliformes*) and *A. forrestii*, and that there were micromorphological grounds for the splitting of *Actinodaphne* s.l. This is further supported by a preliminary molecular study of *Actinodaphne*, which found the genus to be polyphyletic within Laureae (Li et al., 2006). Although Li (1985) and Tsui (1987) had previously regarded *Lindera* sect. *Cupuliformes* as being possibly related to *Litsea* sect. *Cylicodaphne* because of their similar fruit cupules (despite belonging to different genera), this was not supported by our study, in which section *Cylicodaphne* itself was polyphyletic. Furthermore, *Lindera megaphylla* has large leaves aggregated near the top of branchlets (an unusual character in *Lindera*), making it morphologically more similar to some *Actinodaphne* species. *Lindera megaphylla* also bears a pair of pseudo-umbels on each side of an axillary short shoot with a vegetative terminal bud, whereas *A. forrestii* has several sessile pseudo-umbels clustered on an axillary short shoot that also produces a vegetative terminal bud. This suggests that *L. megaphylla* and *A. forrestii* may share a common inflorescence ontogeny and could help to explain why *A. forrestii* was separated from other *Actinodaphne* species in the analyses of Li et al. (2004, 2006) and the current study.

The largely basal and isolated position of *Lindera obtusiloba* (sect. *Palminerviae*) is unusual. This species is deciduous, and its pseudo-umbels are borne in an axillary, mixed bud (leaves and inflorescences together) covered by scales. This kind of mixed bud also occurs in *Sassafras*. According to this character and the later appearance of *L. obtusiloba* in the fossil record, Tsui (1987) suggested that *Lindera* evolved from *Sassafras* during the Miocene, possibly in response to the onset of cooler, more seasonal climates, although this assumes the correct assignment of fossils to extant genera, and generic definition based on vegetative anatomical and morphological features, which is an area of ongoing research.

Neolitsea consistently formed a terminal monophyletic lineage within the *Neolitsea–Actinodaphne* clade, agreeing with the *matK* analysis of Li et al. (2004). The present study (with many more taxa) also shows that *Neolitsea* as a clade is defined by dimerous flowers (apparently a reduction from a trimerous ancestral condition), clustered/verticillate leaf arrangement, triplinerved venation, and decussate, persistent involucral bracts, and that splitting the genus just on leaf venation differences, as suggested as a possibility by Li et al. (2004), is not warranted.

Although two main subclades (*Neolitsea* I and II) were found in all the analyses, the precise composition of these varied, with some taxa moving between them or to a basal grade position depending on the analysis used, possibly due to what is still a relatively small sample size for such a large genus. Similarly, the absence of previously defined morphology-based sections within *Neolitsea* makes "representative" sampling more difficult. As they currently stand, neither *Neolitsea* I nor II has definitive synapomorphies, and they do not differ consistently in leaf venation or inflorescence features. Leaf arrangement seems to be important for two of the larger subclades, with the species in Ia being verticillate, and those of IIb being alternate. Similarly, IIa above *N. penninervis* shows ovoid-ellipsoid fruit, although this feature also occurs in some *Neolitsea* species from subclades I and IIb. Accordingly, the composition of the clades within *Neolitsea* and the nature of any supporting morphological characteristics are the focus of ongoing research.

Similarly, the inclusion of *Actinodaphne paotingensis* inside *Neolitsea* by the ME and ML analyses warrants study. This placement may be related to the instability of the number of floral parts, as there can be six to eight perianth lobes and nine to 15 fertile stamens in this species.

The combined molecular and morphological phylogeny indicates that *Neolitsea* is terminal above *Actinodaphne*, despite previous morphological studies suggesting that *Neolitsea* is closest to *Litsea* (Kostermans, 1957; Hyland, 1989; Rohwer, 1993). Van der Werff (2001) noticed that the inflorescence of *Actinodaphne* is unlike that of other Laureae, and several species (e.g., *A. pilosa* (Lour.) Merr., *A. henryi* Gamble, and *A. obovata* (Nees) Blume) have a paniculate or racemose inflorescence enclosed by imbricate, early deciduous involucral bracts. In our study, the *Neolitsea–Actinodaphne* clade was defined by the possession of verticillate leaves (later becoming terminally clustered in *Neolitsea*) and thyrsoid inflorescences lacking a terminal bud. This latter inflorescence feature is very different from the brachyblast-type short shoot seen in *Laurus* or the *Litsea*, *Lindera*, and *Aperula* clades, which display instead a pseudo-umbellate inflorescence with a vegetative terminal bud and decussate, tardily deciduous involucral bracts that enclose each pseudo-umbel. Rohwer (1993: fig. 87C–E) suggested that reduction from paniculate or racemose inflorescences led to the sessile pseudo-umbellate inflorescences

seen in *Neolitsea*, and this agrees with its position as a derived terminal above *Actinodaphne*. Furthermore, the clustered pseudo-umbels seen in *Actinodaphne* and *Neolitsea* differ from those of *Litsea* or *Lindera*, as the former are thyrsoid, lack terminal buds, and are clustered in leaf axils, whereas the latter are arranged along leafless short shoots and bear a vegetative terminal bud.

Within *Neolitsea*, the separation of *N. ovatifolia* var. *ovatifolia* from variety *puberula* Yen C. Yang & P. H. Huang was unexpected. It may just reflect the widespread distribution and/or regional differentiation within this species, but certainly warrants further study to clarify species and varietal limits in this taxon.

Li (1985) and Tsui (1987) suggested an evolutionary series for the inflorescences in *Litsea* and *Lindera*, and our results concur with their hypotheses. The flowers occur in pseudo-umbels enclosed by decussate involucral bracts and are arranged along a leafless axillary short shoot with a terminal bud that can grow into a normal vegetative branch after flowering. A raceme bearing pseudo-umbels arising from normal growth of the peduncles and growth of the internodes in the short shoot was a synapomorphy for the *Laurus–Litsea–Lindera–Aperula* I clade, with reduction in several distal branches to create fasciculate clusters. In other basal groups, the peduncles and internodes of the short shoot are frequently reduced, so that the flowers are arranged in spikelike pseudo-umbels (the sessile inflorescence condition appears to be plesiomorphic in the Laureae). In *Iteadaphne* and *Dodecadenia*, although they follow this basic racemose pattern, the number of flowers per involucre or pseudo-umbel is reduced to one. In both cases, they seem to represent reduced members of otherwise pseudo-umbellate clades, suggesting that the pattern is convergent.

Previous morphology-based studies recognized considerable variability in both *Litsea* and *Lindera* and variously subdivided them into sections (Bentham, 1880; Hooker, 1890; Li et al., 1984; Tsui, 1987). Hooker (1890) and Li et al. (1984) recognized four sections within *Litsea* (*Litsea*, *Conodaphne*, *Cylicodaphne*, and *Tomingodaphne*) based on habit, leaves, floral characters, inflorescences, and fruit cupules. *Lindera* was similarly divided into eight sections (*Lindera*, *Sphaerocarpae*, *Palminerviae*, *Aperula*, *Cupuliformes*, *Daphnidium*, *Polyadenia*, and *Uniumbellatae*) by Tsui (1987). Although traditional generic delimitations based on two-celled versus four-celled anthers were not supported by our study, several monophyletic subclades are evident that do correspond, in part, to some of these previously recognized sections, and help to shed light on their phylogenetic relationships, as well as character evolution, within the clades. For example, the *Litsea* clade was synapomorphic for four-locular anthers, albeit with *Iteadaphne* embedded within it as a reversal.

Within the *Litsea* clade, *L. glutinosa* (sect. *Litsea* and type species for the genus) and *L. monopetala* (sect. *Conodaphne*) were consistently resolved as sister taxa, with moderate support in the ME and Bayesian analyses. Although clustered on both molecular as well as combined data, the pair is characterized by a lack or incompleteness of the perianth lobes (absent in *L. glutinosa* and early deciduous in *L. monopetala*) (How, 1956).

The *Cylicodaphne* I and II subclades, corresponding to members of *Litsea* sect. *Cyclicodaphne* s.l., were well supported as separate in most of the analyses, and, even in the Bayesian analysis where they formed a single clade, *Cylicodaphne* I was supported and terminal above an unsupported *Cylicodaphne* II grade. Although *L. variabilis* was placed in section *Conodaphne* by Li et al. (1984), it was treated as a member of section *Cylicodaphne* by Hooker (1890) and our results support its return to that section in *Cylicodaphne* I. Within *Cylicodaphne* I, although some species have eight perianth lobes, they all have pseudo-umbellate racemose inflorescences arranged along leafless short shoots, a feature that they share with the *Litsea* subclade (at least in part) and *Iteadaphne*, although there were no morphological synapomorphies to define *Cylicodaphne* I. In *Cylicodaphne* II, the species have one to several clustered pseudo-umbels on an axillary short shoot and ovoid-elliptical fruits, supporting the inclusion of *Dodecadenia*.

Lindera fruticosa (sect. *Sphaerocarpae*) was related to *Iteadaphne* and section *Daphnidium* in the SW analysis, despite its possession of an umbel with an elongate peduncle and its deciduous habit. Nevertheless, it was placed as a less-derived member of the *Lindera* clade in the ME and ML analyses, with which it shares a deciduous habit and possession of a single or few pseudo-umbels clustered on the short shoot. Its basal position may be related to the intermediate situation of leaf venation, as it can possess both triplinerved and pinninerved leaves. However, as none of our analyses showed bootstrap support for the position of this taxon, its associations must be regarded with caution for the present, pending further studies.

The *Lindera* clade consists of species from section *Lindera* and members of *Litsea* sections *Conodaphne* and *Tomingodaphne*. In deciduous habit, *Lindera kariensis* W. W. Sm. (sect. *Lindera*) is very like *Litsea sericea* (Wall. ex Nees) Hook. f. (sect. *Tomingodaphne*), except for the difference in number of anther

cells. Nevertheless, the placement of the evergreen *Litsea umbellata* (Lour.) Merr. (sect. *Conodaphne*) within the group is unusual, as all other members of this clade are deciduous. *Litsea umbellata* also appears unusual within the clade, as its cupule bears persistent tepals. Nevertheless, it is possible that deciduous species that bear cupules without persistent tepals may have evolved from evergreen ancestors with persistent cupule tepals, and in section *Conodaphne* s. str., there are species with and without persistent cupule tepals. Similarly, section *Tomingodaphne* contains two vegetative terminal shoot bud forms in the axillary short shoots: naked versus scaly (Hooker, 1890).

Rohwer (1993) and van der Werff and Richter (1996) recognized a large portion of what we now regard as the Laureae, including *Actinodaphne*, *Dodecadenia*, *Iteadaphne*, *Lindera*, *Litsea*, *Neolitsea*, *Parasassafras*, and *Sinosassafras*. Although *Sassafras* and *Umbellularia* were also included, they should be removed based on the results of Chanderbali et al. (2001), Li et al. (2004), and the present study. This is further supported by the possession of racemose inflorescences in *Sassafras* and the presence of bisexual flowers with extrorse anther cells in the third whorl in *Umbellularia*.

In our study, we sampled species representing all genera and most sections in the core Laureae, and it is clear that a major disparity exists between our molecular phylogenetic results and more traditional morphology-based taxonomic concepts of generic and infrageneric classification in the tribe. For example, both current and earlier analyses (Li & Christophel, 2000; Li et al., 2004) confirm that using two- versus four-celled anthers to separate *Litsea* s.l. from *Lindera* s.l. results in polyphyletic or paraphyletic genera (Rohwer et al., 1991; Rohwer, 1993; van der Werff & Richter, 1996; Li & Christophel, 2000; Li et al., 2004). This character should, therefore, be used with caution in Laureae classifications, although it does seem to be useful for defining some of the higher-level clades in the group (e.g., the *Litsea* clade s. str.).

The significance of other traditional characters often used at the generic level, such as dimerous or trimerous flowers, also needs re-evaluation. For example, *Laurus* and *Neolitsea* have been related previously based on their dimerous flowers, but not in our analyses. Similarly, the nature and arrangement of basal inflorescence involucral bracts (i.e., early deciduous vs. tardily deciduous; imbricate vs. decussate) are traditionally important characters for delimiting genera. However, whereas *Actinodaphne*, *Parasassafras*, and *Sinosassafras* can be distinguished from other Laureae by the possession of early deciduous, imbricate bracts, these character states are convergently homoplasious in our analyses. This suggests that although potentially diagnostic for relating the latter two genera, the character is of limited phylogenetic value, especially given the position of *Actinodaphne* as a basal grade below *Neolitsea*.

Nevertheless, the clades identified in this study do provide opportunities to examine evolution of specific morphological characters in Laureae, a task already initiated by Li and Christophel (2000), and of particular interest will be studies of inflorescence development. The most common inflorescence form in Laureae is pseudo-umbels clustered in the leaf axils, but our analyses show that there are apparently at least two different ways to produce this structure. One, suggested by previous studies (Li, 1985; Tsui, 1987) and seen in *Laurus* and the *Litsea*, *Lindera*, and *Aperula* I and II clades, results from shortening of the internodes in the short shoot with a vegetative terminal bud. This vegetative terminal bud may be normal and scaly or naked, well developed and large, poorly developed, or even reduced. Sometimes the terminal vegetative shoot along with one or two lateral fertile short shoots with pseudo-umbels merge to form a single axillary mixed bud, and the peduncle of pseudo-umbels and the internodes of the short shoot may be developed or reduced. Given the positions of these taxa toward the base of the trees, this feature appears to be plesiomorphic in Laureae.

The second, derived condition, seen in the *Neolitsea–Actinodaphne* clade, results instead from shortening of a thyrsoid inflorescence axis and lacks a vegetative terminal bud. However, given the lack of deep-branch support for the major lineages in this study, definitive conclusions about the phylogeny of inflorescence ontogeny must await further studies.

In conclusion, our study indicates the need for caution in the use of morphological similarity for assessing affinities between taxa in the Laureae. Traditional characteristics of habit, leaf venation, inflorescence, and floral structure appear in many cases to have been the result of convergent and/or parallel evolution and, therefore, may not be indicative of evolutionary affinity or useful for taxon delimitation at higher levels. Added to this is the possibility that some features such as pseudo-umbels may not be homologous. Nevertheless, the study identifies areas in which future research may help to clarify or correct problems of homology and ontogenetic convergence. It provides a hypothesis for possible phylogenetic relationships in Laureae, albeit based on a single, rapidly evolving genome, and gives direction for future studies using multiple independent and possibly more conservative markers to assess the phylogenetic hypotheses that our results indicate.

This will provide the foundation for a revised phylogeny-based classification of the Laureae in which reliable synapomorphies are backed by data from a range of sources.

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COMPARATIVE POLLINATION BIOLOGY OF SYMPATRIC AND ALLOPATRIC ANDEAN *IOCHROMA* (SOLANACEAE)[1]

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Abstract

Field studies were conducted for 15 species of *Iochroma* Benth. and the nested genus *Acnistus* Schott to quantify the diversity of pollination systems and to assess the potential contribution of pollinator behavior to the persistence of closely related species in sympatry. We combined measures of pollinator visitation and pollen deposition to estimate the importance of major groups of pollinators for each species, and we calculated proportional similarity in the pollinator assemblage among species. We found that 12 species of *Iochroma*, encompassing a range of flower colors and sizes, were principally pollinated by hummingbirds and, in many cases, by the same hummingbird species. The remaining species were either pollinated by a mix of hummingbirds and insects (two species) or exclusively by insects (two species). Based on proportional similarity values, the overlap in pollinator assemblages was found to be higher for sympatric species than for allopatric ones, reflecting sharing of local pollinator fauna. However, observations of individual pollinator fidelity, perhaps related to territorial interactions among hummingbirds, suggested that pollinators may still contribute to the reproductive isolation of sympatric congeners. Nonetheless, because interspecific pollen flow does occur, the maintenance of species boundaries in sympatry probably requires postmating reproductive isolating mechanisms.

Key words: *Acnistus*, flower color, hummingbird pollination, *Iochroma*, pollen deposition, pollinator importance, pollinator visitation, reproductive isolation, sympatry.

The rich floristic diversity of the Neotropics has often been attributed to the complex and specialized interactions between plants and animals, typically in the form of herbivory or pollination (Faegri & van der Pijl, 1966; Janzen, 1973; Johnson & Steiner, 2000). For example, Gentry (1982) noted that the Andean-centered families, such as the Ericaceae, Gesneriaceae, and Campanulaceae, which account for a large proportion of the Neotropical species diversity, are biotically pollinated and often appear to be specialized for particular groups of animals such as hummingbirds or bats (Perret et al., 2001; Luteyn, 2002; Muchala, 2006). While geographical patterns in pollinator specialization have become the subject of debate (Ollerton & Cranmer, 2002; Olesen & Jordano, 2002), the largest barrier to understanding the role of pollinators in the diversification of Neotropical taxa remains the paucity of detailed studies of pollination ecology, particularly those that catalog not only the range of visitors but their effectiveness as pollinators (Kay & Schemske, 2004).

Here, we investigate the pollination biology of *Iochroma* Benth., an Andean genus of approximately 25 species of Solanaceae (Smith & Baum, 2006). Several authors (e.g., Lagerheim, 1891; Cocucci, 1999) have speculated that the showy tubular flowers of *Iochroma* species are pollinated by hummingbirds; however, no previous field studies of pollination exist for this group. While several *Iochroma* species (e.g., *I. fuchsioides* (Humb. & Bonpl.) Miers and *I. gesnerioides* (Kunth) Miers) are indeed a close fit to the classic hummingbird syndrome flower, namely red, scentless, and tubular, most species vary from this suite of traits, suggesting pollination by other groups

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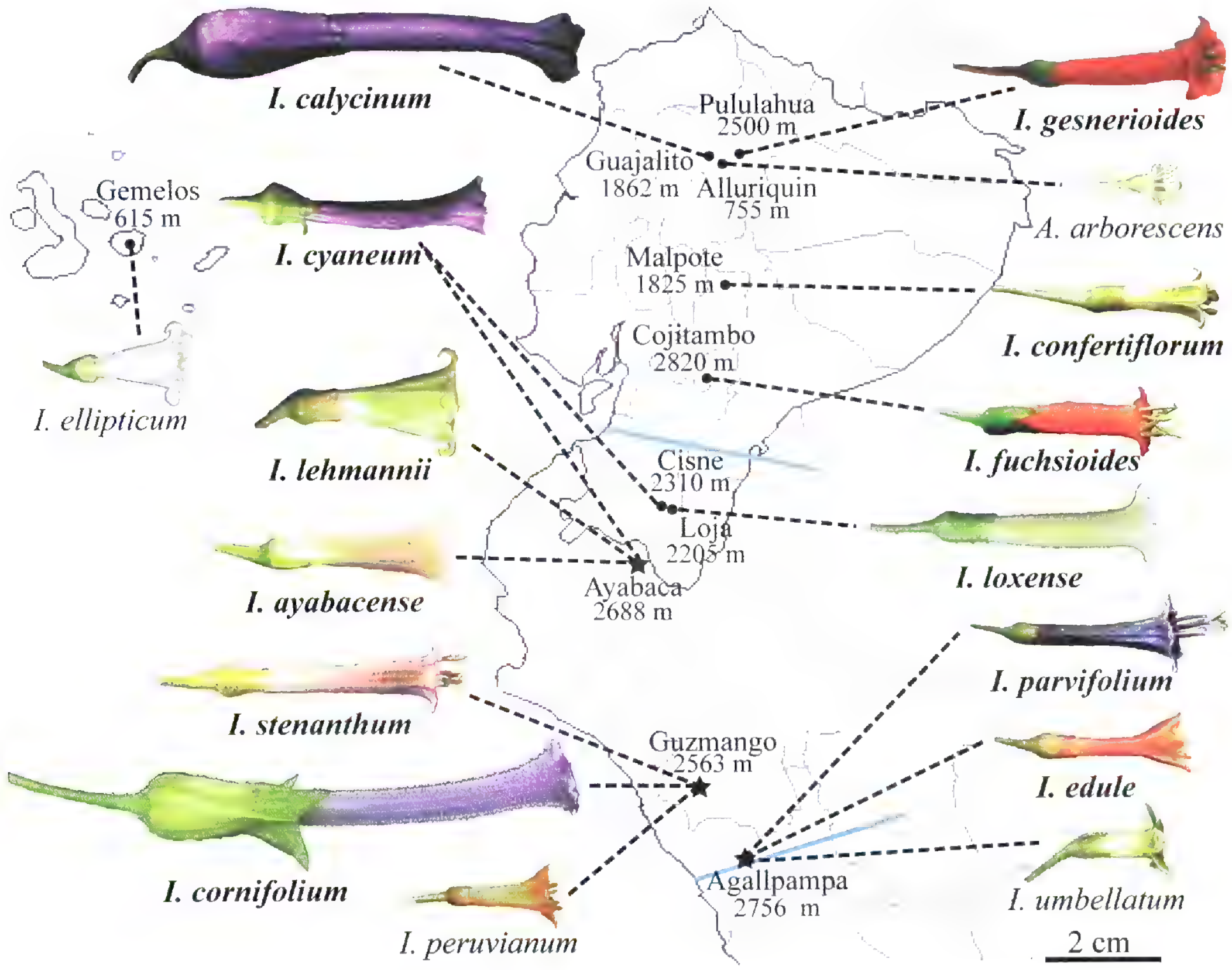

Figure 1. Study sites in Ecuador and Peru in northwestern South America. Country borders are in dark grey, and provinces or departments are bounded in light grey. Approximate boundaries of the Amotape–Huancabamba zone (from Weigend, 2002) are indicated with blue lines. Galápagos Islands (upper left) are not to scale. Study species at each site are indicated with dashed lines, and flowers are shown to scale (bottom right). Sites with sympatric taxa are marked with stars, and the names of the principally hummingbird-pollinated taxa (as determined by this study) are in boldface.

of animals (Fig. 1). For instance, the flowers of *I. confertiflorum* (Miers) Hunz. are greenish white, tubular, and scented, traits more commonly associated with moth pollination (Faegri & van der Pijl, 1966). Also, many species exhibit the peculiar combination of long, tubular, blue or purple flowers, a combination that does not correspond to any known syndrome (Faegri & van der Pijl, 1966). Given the diversity of floral morphologies present in *Iochroma*, we predicted that the composition of visitors and their effectiveness as pollinators would vary substantially between species.

Differences in pollination system among *Iochroma* species may carry important implications for the maintenance of species boundaries. In some parts of the Andes, *Iochroma* species occur in sympatry and flower together during the rainy season, creating the potential for interspecific pollen flow. Although some hybrids have been documented in zones of sympatry (Smith & Baum, 2006), interspecific hybridization is not rampant, and many taxa coexist with no observed hybrid formation (Smith, 2006). Pollinators may contribute to reproductive isolation in areas of sympatry either by forming specialized relationships with particular *Iochroma* species or by exhibiting constancy during foraging bouts, such that interspecific gene flow is limited (Jones, 1978; Campbell & Motten, 1985; Waser, 1986).

In the present study, we assessed the specialization of pollinators on 15 *Iochroma* species and the nested genus *Acnistus* Schott, and, in areas of sympatry, we examined the importance of pollinator behavior in interspecific pollen flow. In order to characterize the pollination system for each species, we measured pollinator visitation rates and pollen deposition for four major groups of pollinators (hummingbirds [Trochilidae], as well as hymenopteran, lepidopteran, and dipteran insects), and we calculated a composite variable, pollinator importance, for each group. Using the visitation rates for each pollinator, we compared the similarity in pollinator assemblage for allopatric and sympatric species pairs to determine if sympatric

taxa tend to show more divergence in their pollinator use than is typical for allopatric taxa. Finally, we compiled observations of individual pollinator movements in sympatric areas to examine the possibility of preferential visitation by individual pollinators.

Materials and Methods

Study Taxa

Our taxon sampling of *Iochroma* represented the range of floral variation within the genus. *Iochroma* flowers may be red, orange, yellow, white, green, blue, and purple, and all of these colors were included in our study group. Flower form in *Iochroma* ranges from campanulate to narrowly tubular. Here, the campanulate form is represented by *Acnistus arborescens* (L.) Schltdl., the sole member of the genus *Acnistus* (Hunziker, 2001), which is nested within the core clade of *Iochroma* (Smith & Baum, 2006). Corolla tube length varies more than eight-fold in *Iochroma*, and in our study taxa ranges from less than 1 cm to more than 6 cm (Fig. 1). Only two *Iochroma* species (*I. ellipticum* (Hook. f.) Hunz. and *I. confertiflorum*) produce any noticeable scent, and both were included here. Their light, sweet scent is very similar to that of their close relative *A. arborescens*, whose scent apparently derives from a mixture of 3,5-dimethoxytoulene, jasmine, anisaldehyde, and methyl anthranilate (Kaiser, 2000).

The selection of study taxa also took into account the recent phylogenetic analysis of Iochrominae (Smith & Baum, 2006), which indicated that the genus *Iochroma* is not monophyletic, but is instead divided among three major clades. Eleven of the studied taxa fall into the large core clade of 13 *Iochroma* species (Smith & Baum, 2006), which also includes *Acnistus arborescens*. Outside of this core group of iochromas, we sampled two additional species, *I. parvifolium* (Roem. & Schult.) D'Arcy and *I. umbellatum* (Ruiz & Pav.) Hunz. ex D'Arcy (Smith & Baum, 2006). Also, we studied two recently named taxa, *I. ayabacense* S. Leiva and *I. stenanthum* S. Leiva, Quipuscoa & N. W. Sawyer, which appear to be of hybrid origin (Smith & Baum, 2006). Phylogenetic analyses have suggested that *I. ayabacense* is a hybrid between *I. lehmannii* Bitter and *I. cyaneum* (Lindl.) M. L. Green, and *I. stenanthum* is probably a hybrid between *I. cornifolium* (Kunth) Miers and *A. arborescens* (Smith & Baum, 2006). Vouchers were collected for all studied populations; these are given in Appendix 1.

Study Sites

Iochroma species are mainly distributed in the Andes of Colombia south to Peru, where they occur as sparsely distributed large shrubs or treelets in scrub or cloud forest between 2200 and 2900 m. The greatest species richness occurs in the Amotape–Huanacamba zone at the border of Ecuador and Peru (Smith & Baum, 2006; Fig. 1). Whereas outside of this zone *Iochroma* species are typically found in allopatry, geographic ranges frequently overlap within the Amotape–Huanacamba zone, and up to four species can co-occur within a single 1-km^2 area. A few species pairs (e.g., *I. cyaneum* and *I. cornifolium*) hybridize in areas of contact, but many do not (e.g., *I. umbellatum* and *I. edule* S. Leiva) (Smith, 2006). There are some differences among taxa in microhabitat preferences (e.g., soil, light, and moisture conditions), but generally, *Iochroma* species are found in areas of moderate disturbance, such as forest gaps, trails, dry stream beds, or field edges. The 11 study sites were typically located on the outskirts of rural towns or villages, in areas of mixed secondary vegetation and small-scale agriculture; the locations are shown in Figure 1 and listed in Appendix 1.

Iochroma species flower throughout the year, but peak flowering occurs during the rainy season, roughly from December to April in the Andean regions of Ecuador and Peru where these studies took place. Flowering peaks slightly earlier in northern Ecuador (December) than in southern Ecuador and Peru (January to February). During these months, the weather is typically sunny in the morning and cloudy or rainy in the afternoon and evening. Pollinator observations for each species were made over a three- to four-day period during the rainy season; a few studies were extended for a fifth day when extremely rainy conditions persisted for several days or when the overall rate of pollinator visitation was low. The dates of each study are given in Appendix 1. To the extent possible, the pollination studies were conducted in sites that were well within the geographic and altitudinal range of the species and contained many large, flowering individuals (20 to 200). Up to 14 individuals in each population were incorporated into each study (Appendix 1), although the scarcity and/or poor accessibility of some taxa limited the number of available individuals (e.g., four plants for *I. peruvianum* (Dunal) J. F. Macbr.).

Floral Biology

Stigma receptivity was judged using the hydrogen peroxide test (Kearns & Inouye, 1993). Styles were collected from flowers throughout anthesis (from bud to a wilted flower) and dipped in hydrogen peroxide. The youngest stigma to yield a positive result (production of bubbles) was taken to indicate the onset of stigma receptivity.

Nectar volume was measured with calibrated glass micropipettes, and the percentage of sugar was estimated using a temperature-compensated hand refractometer (QA Supplies, Norfolk, Virginia, U.S.A.) accurate to 0.2% between 0% and 32% sugar by volume. Nectar was extracted by pressing the micropipette into the spaces between the filaments where the liquid accumulates in *Iochroma*. Samples were taken from first- or second-day flowers (covered with 1-mm mesh bags before anthesis) at 3-hr. intervals from 0700 to 1900 hr. Each flower was only sampled once. Flowers were sampled from five to 10 individuals per study population.

VISITATION

Pollinator visits were recorded for two to three days during three observation periods: morning (0600 to 0900 hr.), midday (1100 to 1400 hr.), and evening (1700 to 2000 hr.). Two hours of observations were recorded during each period, but with the time required to move between study individuals, each period spanned 3 hr. The time periods were chosen based on previous observations that suggested that these are periods of high pollinator activity. During the studies, the sun rose at ca. 0600 hr. and set at 1900 hr. Observations were made while sitting 2.5 to 3.5 m from a subject plant to minimize the distraction of visitors. Both the number of visits to the subject plant and the number of legitimate flower visits (sensu Jones & Reithel, 2001) were noted. Bird visitors were identified to the lowest possible taxonomic level using field guides and consultation with experts. Visiting insects were collected in ethanol (70%) and later identified by comparing them to reference collections at the University of Wisconsin–Madison, the University of Illinois–Urbana, and the Charles Darwin Research Station. All insect specimens were deposited in the University of Wisconsin–Madison Insect Research collection.

Plant and flower visitation rates were calculated from raw observation data by dividing the total number of visits by a given pollinator by the time observed (in flower hours to correct for differences in display size among individuals, sensu Dafni, 1992). Flower hours were calculated from a series of observations (1 to n) as follows: $FH = (D_1 \times T_1) + (D_2 \times T_2) + \ldots (D_n \times T_n)$, where FH equals the number of flower hours, D is the display size (number of flowers on the plant), and T equals the number of hours the plant was observed. Visitation rates were calculated both for individual species and for four major groups of pollinators (hummingbirds, as well as hymenopteran, lepidopteran, and dipteran insects). Flower visitation rates were used to compute the proportional similarity (PS) of pollinator assemblages between all pairs of *Iochroma* species (Schemske & Brokaw, 1981; Kay & Schemske, 2003). This measure takes into account both the number of pollinator species shared and their visitation frequency; PS for a pair of plant species is $1 - \frac{1}{2} \Sigma |P_{ai} - P_{bi}|$, where P_{ai} and P_{bi} are the proportions of the total visitation rate made up by pollinator species i for plant species a and b, and differences in P_{ai} and P_{bi} are summed across all pollinator species, 1 ... i. PS values range from 0 to 1, with higher values indicating greater overlap in the pollinator visitation between two species.

INTERSPECIFIC POLLINATOR MOVEMENTS

We assessed the potential for interspecific pollen flow in sympatry by observing pollinator visitation to individual plants from different species growing side by side. The closest pairs of plants, typically 1 to 5 m apart, were selected for observation, and movement of the pollinators within the plants and between the plants was recorded. We examined the visitation patterns for bias toward particular *Iochroma* species by a given pollinator by comparing the expected to the observed number of visits with χ^2 analysis (as in Schemske, 1981). Expected values are the number of visits expected if visits are directly proportional to display size. Also, using the observations of the sympatric plant pairs, we compared the number of plant visits that involved movement between species to those that were restricted to one species as an additional measure of pollinator fidelity.

POLLEN DEPOSITION AND POLLINATOR IMPORTANCE

To measure pollen deposition by different pollinator species, virgin flowers (covered with green or black 1-mm mesh bags before anthesis) were presented to pollinators and then re-bagged after a single visit by a single pollinator. The pollen loads on visited stigmas were compared to stigmas from flowers that were bagged for the duration of the experiment to determine if these species require biotic pollination. Also, we collected and examined stigmas from unbagged flowers to assess the typical pollen deposition for flowers exposed to unlimited visits.

To test for nocturnal pollination, stigmas were collected from flowers that had been bagged during the day but left unbagged at night (1900–0600 hr.). Additionally, for 13 study species (excluding *Acnistus arborescens*, *Iochroma cyaneum*, and *I. gesnerioides*), stigmas were collected from flowers that were bagged at night but unbagged during the day (0600–1900 hr.). Where there were sufficient flowers, all four treat-

ments (always bagged, never bagged, bagged during night only, bagged during day only) were completed for each individual. Styles were removed from flowers that were already open at the beginning of the experiment to ensure that only virgin flowers were included.

Styles from visited or treated flowers were collected 6 to 12 hr. after replacement of the bag, fixed in formalin, acetic acid, and alcohol (FAA) for 12 to 24 hr. and transferred to ethanol (70%) for storage. The fixed stigmas were examined for the presence of germinating pollen grains using a modified version of Martin's (1959) protocol. The styles were soaked in 1M NaOH to soften (10 min.), washed three times in 50 mM KPO_4 buffer (5 min. per wash), stained with 0.05% decolorized aniline blue in 50 mM KPO_4 buffer for 5 min., squashed on a microscope slide, and viewed under ultraviolet fluorescence microscopy. When the style contained fewer than ca. 100 grains, all were counted. When there were greater than 100 grains, the style was divided into sections (e.g., halves, quarters), and a count from a representative section was used to estimate the total load. Non-fluorescing grains (not germinating and/or not Solanaceae) were excluded from counts. *Iochroma* species do not differ markedly in pollen morphology, so it was not possible to identify pollen to species. To relate pollen load to ovule availability, the average number of ovules per flower in each species was estimated by counting ovules from enough flowers to bring the standard error to less than 10% of the mean, when sufficient flowers were available.

Pollen deposition (quality) was combined with visitation rate (quantity) to give an overall estimate of pollinator importance (Waser & Price, 1983; Schemske & Horvitz, 1984; Herrera, 1987; Mayfield et al., 2001). Here, the importance of each group of pollinators was calculated as the product of the relative visitation rate and the proportion of available ovules potentially pollinated by a single visit (using the previously described estimates of ovules per flower). If the average number of pollen grains deposited by a single visit exceeded the estimated number of ovules, the proportion was set to 1.0. We used this scaled deposition for two reasons. First, it allowed us to take into account the differences in ovule number and, thus, in potential per-visit effectiveness across study species. Second, this approach accommodates the fact that seed set typically levels off quickly with increasing pollen load (Silander & Primack, 1978; Kohn & Waser, 1985), so pollen deposition that greatly exceeds the number of ovules (as observed here for many hymenopterans) is unlikely to result in a proportional increase in fitness.

Results

Floral Biology

Measurements of stigma receptivity showed that *Acnistus* and all species of *Iochroma*, except *I. umbellatum*, are protogynous. Stigmas are fully receptive when the flowers open, and the anthers dehisce 1 to 3 hr. later except in *I. umbellatum*, in which the anthers are already dehisced when the flower opens. *Iochroma* flowers open asynchronously throughout the day, but with some tendency toward opening in the morning, and they do not close at night. The stigmas remained receptive until the flowers wilted, two to three days after opening.

Nectar-standing crop varied widely across taxa, but did not show strong diurnal patterns, in large part because of the staggering of flower maturation during the day. For comparison across taxa, we pooled measurements from all sampling times and computed averages for nectar volume and concentration (Table 1). The small-flowered *Acnistus arborescens* produced the lowest volume of nectar (0.5 ± 0.1 µl) and presented the smallest reward, both on a per-flower and a per-plant basis. The large-flowered *Iochroma calycinum* Benth. produced the most nectar (38.4 ± 3.0 µl), although it did not offer the highest per-flower reward because of its low sugar concentration (14.5 ± 0.3%). The most rewarding species was *I. loxense* (Kunth) Miers due to its high nectar volume (37.0 ± 2.7 µl), high sugar concentration (24.0 ± 0.5%), and large display size (120.3 ± 38.1 flowers per plant).

Visitation

Over the course of ca. 264 hr. of observation, 47 pollinators were observed legitimately visiting the 16 study species (Appendix 2). Bees and species of flower-piercing birds (*Diglossa* Chapman [Thraupidae]) were frequent illegitimate visitors, robbing nectar by making holes in the sides of the corollas. These illegitimate visits were not included in visitation rates. Legitimate visitors included hummingbirds (Apodiformes, Trochilidae) and a wide variety of insects from the Hymenoptera, Diptera, and Lepidoptera (Appendix 2).

Hummingbird visits were evenly spread across all observation periods, hymenopteran visits were most common in the midday period (1100–1400 hr.), dipteran visits occurred primarily in the morning (0600–0900 hr.), and lepidopteran visits (mainly moths) were most frequent in the evening period (1700–2000 hr.). Hymenoptera and Diptera visited 2.6 and 2.1 flowers per plant visit, respectively, whereas Lepidoptera visited 4.5 flowers on average and hummingbirds 15.8 flowers.

Table 1. Nectar rewards across study taxa.[1]

Species	Flowers sampled	Average nectar volume (μl) ± SE	Average percent sugar ± SE	Average reward per flower[2] ± SE	Average reward per plant[3] ± SE
Acnistus arborescens	16	0.5 ± 0.1	14.4 ± 2.5	0.1 ± 0.02	2.3 ± 0.6
Iochroma ayabacense	25	11.8 ± 1.0	22.5 ± 0.3	2.7 ± 0.2	459.9 ± 128.1
I. calycinum	26	38.4 ± 3.0	14.5 ± 0.3	5.7 ± 0.5	169.3 ± 63.9
I. confertiflorum	37	17.5 ± 1.6	20.9 ± 0.6	3.8 ± 0.4	424.4 ± 148.6
I. cornifolium	20	37.3 ± 3.2	18.4 ± 0.3	6.9 ± 0.6	603.0 ± 121.8
I. cyaneum	29	17.7 ± 1.9	23.1 ± 0.7	4.1 ± 0.4	177.8 ± 42.6
I. edule	28	10.9 ± 1.1	20.7 ± 0.4	2.3 ± 0.2	704.7 ± 167.6
I. ellipticum	29	1.8 ± 0.3	7.6 ± 1.4	0.2 ± 0.05	10.22[4]
I. fuchsioides	32	20.2 ± 1.8	26.4 ± 0.7	5.2 ± 0.4	414.8 ± 79.7
I. gesnerioides	11	10.0 ± 2.4	16.3 ± 0.66	1.7 ± 0.4	841.7 ± 482.2
I. lehmannii	26	7.0 ± 0.6	27.2 ± 0.84	1.8 ± 0.2	515.3 ± 229.6
I. loxense	30	37.0 ± 2.7	24.0 ± 0.47	8.7 ± 0.5	1048.1 ± 338.6
I. parvifolium	17	17.5 ± 1.1	20.0 ± 0.7	3.5 ± 0.3	342.6 ± 133.7
I. peruvianum	20	6.9 ± 0.7	21.3 ± 0.6	1.4 ± 0.2	122.6 ± 29.0
I. stenanthum	23	21.4 ± 2.5	19.5 ± 0.4	4.2 ± 0.5	880.5 ± 243.7
I. umbellatum	32	2.2 ± 0.4	16.4 ± 0.6	0.4 ± 0.08	31.1 ± 17.1

[1] All values are shown with standard error (SE).
[2] Reward per flower is the product of volume per flower and percent sugar.
[3] Reward per plant is the product of average reward per flower and average display size (Appendix 1).
[4] Variation in display size across the population was not measured in *Iochroma ellipticum*, thus no standard error was calculated.

Based on relative flower visitation, we classified the species into three broad classes: principally hummingbird pollinated, mixed hummingbird/insect pollinated, and exclusively insect pollinated (Table 2). Taxa for which greater than 75% of the total visitation was concentrated on a single group of pollinators were considered to be specialized for that group (Fenster et al., 2004). With this criterion, 11 of the study species were considered to be principally hummingbird pollinated (Table 2). *Iochroma calycinum* had only 65% hummingbird visitation, but was also classified as principally hummingbird pollinated because most insect visitors were found to be poor pollen vectors (described below; Table 3). Two species, *I. peruvianum* and *I. umbellatum*, were visited almost equally by hummingbirds and hymenopterans and were considered to have a mixed bird and insect pollination system. *Acnistus arborescens* and *I. ellipticum* were visited frequently by all three groups of insects but no hummingbirds were observed (Table 2), so these two taxa were considered insect pollinated. Thus, we did not observe a diverse array of specialized systems, at least at the level of major pollinator groups.

Iochroma species appeared more divergent in pollination system at the pollinator species level, but these differences may be driven more by geography than by specialization for certain pollinators. As described previously, we calculated the pairwise PS metric from the pollinator visitation rates (Appendix 2) and used these values to assess the overlap in pollinator assemblage among species (Fig. 2). We observed that species with different pollination systems (Table 2) did not have significantly lower proportional similarity than species with the same pollination systems (mean PS = 0.13 ± 0.02 vs. 0.18 ± 0.03, unpaired *t*-test: $P = 0.16$). That is, two principally hummingbird-pollinated species did not necessarily show greater overlap in pollinator assemblage than a principally hummingbird-pollinated and an insect-pollinated species. However, species studied in allopatry had lower PS than those in sympatry (mean PS = 0.13 ± 0.02 vs. 0.51 ± 0.10; $P < 0.00001$) and species living in different regions had significantly lower proportional similarity than those living in the same region (mean PS = 0.08 ± 0.01 vs. 0.36 ± 0.04; $P < 0.00001$).

INTERSPECIFIC POLLINATOR MOVEMENTS

To better understand pollinator activity in areas of sympatry, we examined patterns of pollinator visitation to pairs of plants from different sympatric species. Our observations revealed biased patterns for most pollinator species. We observed that the larger hummingbirds (e.g., *Coeligena iris* Gould and *Colibri coruscans* Gould) visited the species that provided the greater nectar reward significantly more frequently, in every case with a sufficient sample size (Table 4). The smaller hummingbirds *Adelomyia melanogenys* Fraser, *Myrtis fanny* Lesson, and *Polyonymus caroli* Bourcier often showed

Table 2. Plant and flower visitation rates across study taxa.[1,2]

	All visitors		Trochilidae		Hymenoptera		Lepidoptera		Diptera		Principal pollinators
	Plant	Flower	Plant	Flower	Plant	Flower	Plant	Flower	Plant	Flower	
Acnistus arborescens	0.130	0.428	0	0	0.024	0.082	0.010	0.091	0.096	0.255	insects
Iochroma ayabacense	0.010	0.186	0.010	0.186	0	0	0	0	0	0	birds
I. calycinum	0.050	0.140	0.008	0.090	0.017	0.024	0	0	0.025	0.025	birds
I. confertiflorum	0.037	0.221	0.011	0.173	0.024	0.042	0.002	0.006	0	0	birds
I. cornifolium	0.016	0.185	0.012	0.177	0.004	0.008	0	0	0	0	birds
I. cyaneum	0.075	0.299	0.041	0.238	0.027	0.040	0.008	0.021	0	0	birds
I. edule	0.012	0.177	0.011	0.175	4×10^{-4}	0.001	2×10^{-4}	0.001	0	0	birds
I. ellipticum	0.018	0.022	0	0	0.007	0.008	0.007	0.008	0.005	0.005	insects
I. fuchsioides	0.032	0.304	0.008	0.235	0.020	0.049	0.003	0.020	0	0	birds
I. gesnerioides	0.008	0.165	0.006	0.148	0.001	0.010	0.001	0.006	0	0	birds
I. lehmannii	0.007	0.341	0.006	0.338	0.001	0.003	0	0	0	0	birds
I. loxense	0.040	0.283	0.017	0.251	0.023	0.032	0	0	0	0	birds
I. parvifolium	0.023	0.336	0.016	0.309	0.007	0.027	0	0	0	0	birds
I. peruvianum	0.033	0.107	0.008	0.047	0.025	0.060	0	0	0	0	birds/insects
I. stenanthum	0.012	0.263	0.010	0.259	0.002	0.004	0	0	0	0	birds
I. umbellatum	0.034	0.298	0.008	0.154	0.022	0.140	0.001	0.001	0.002	0.003	birds/insects
All species	0.032	0.232	0.011	0.174	0.012	0.032	0.002	0.009	0.008	0.017	

[1] Rates are presented as the numbers of visits per plant or flower per flower hour (see Methods, Visitation).
[2] Pollinator groups are hummingbirds (Trochilidae) and the three orders of insects observed.

Table 3. Pollen deposition by pollinators and during treatments.[1,2]

	Trochilidae		Hymenoptera		Lepidoptera		Diptera		Day control[3]		Night control[3]		Bagged control		Unbagged control	
	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size
Acnistus arborescens			898 ± 128	11	266 ± 118	5	149 ± 59	22	*		718 ± 241	4	0 ± 0	4	811 ± 204	3
Iochroma ayabacense	264 ± 56.0	21							285 ± 149	5	17 ± 12	7	1 ± 1	8	414 ± 89	13
I. calycinum	579 ± 428	3	2248 ± 727	4			139 ± 11	2	2058 ± 322	11	60 ± 38	8	134 ± 83	5	1208 ± 170	14
I. confertiflorum	971 ± 217	7	389 ± 75	4	212 ± 106	3			1262 ± 178	17	111 ± 48	19	70 ± 45	7	1462 ± 178	12
I. cornifolium	1436 ± 215	3	1558 ± 603	7					1396 ± 331	27	910 ± 193	5	152 ± 33	6	2209 ± 242	27
I. cyaneum	1076 ± 332	7	732 ± 720	2	88	1			*		115 ± 77	14	24 ± 11	14	775 ± 110	26
I. edule	1290 ± 240	22	2355 ± 137	2	2144 ± 944	2			2422 ± 404	16	878 ± 312	11	1 ± 1	6	1650 ± 140	28
I. ellipticum			97 ± 51	3	1326 ± 387	5	257 ± 78	2	860 ± 151	16	534 ± 174	11	29 ± 13	10	1769 ± 464	6
I. fuchsioides	279 ± 194	7	1151 ± 343	4	317 ± 199	4			1447 ± 265	13	0 ± 0	10	2 ± 2	8	1699 ± 177	19
I. gesnerioides	1107 ± 791	3	2780 ± 301	11	†				*		1 ± 1	5	8 ± 4	19	1423 ± 114	33
I. lehmannii	3083 ± 577	11	594 ± 594	2					4155 ± 853	10	122 ± 119	4	23 ± 13	4	4367 ± 487	14
I. loxense	407.1 ± 259	5	447 ± 205	6					804 ± 142	21	238 ± 91	12	2 ± 2	16	790 ± 106	28
I. parvifolium	14 ± 7	5	720	1					109 ± 52	9	17 ± 11	8	0 ± 0	13	438 ± 86	27
I. peruvianum	1545	1	3328 ± 334	4					2614 ± 608	9	173 ± 151	5	142 ± 65	6	1956 ± 473	12
I. stenanthum	166 ± 59	9	1014	1					761 ± 237	14	263 ± 181	6	1 ± 1	5	686 ± 114	26
I. umbellatum	58	1	727 ± 493	2	†		0	1	438 ± 255	5	365 ± 177	6	17 ± 17	13	937 ± 132	22
All taxa	940 ± 121	105	1472 ± 157	64	712 ± 194	20	151 ± 49	27	1453 ± 122	154	262 ± 42	135	28 ± 6	144	1323 ± 67	310

SE = standard error.

[1] For each plant species, the average number of pollen grains deposited by each group of visitors is presented; blank cells indicate that the pollinator group was not observed visiting that plant species.

[2] The sample size is the number of visited stigmas counted for each pollinator group or treatment.

[3] Day control refers to flowers unbagged only during the day, and night control to those unbagged only during the night.

* Treatment not completed for this species.

† Although this class of visitor was recorded for that species, no pollen counts were obtained.

	Species (site)	*Acnistus arborescens* (ALL)	***I. calycinum*** (GUA)	***I. gesnerioides*** (PUL)	***I. confertiflorum*** (MAL)	***I. fuchsioides*** (COJ)	***I. cyaneum*** (CIS)	***I. loxense*** (LOJ)	***I. edule*** (AGA)	***I. parvifolium*** (AGA)	*I. umbellatum* (AGA)	***I. ayabacense*** (AYA)	***I. lehmannii*** (AYA)	*I. peruvianum* (GUZ)	***I. cornifolium*** (GUZ)	***I. stenanthum*** (GUZ)
	Iochroma ellipticum (GAL)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. ECU	*Acnistus arborescens* (ALL)		0	0.06	0.02	0.09	0	0.01	0.01	0.08	0.11	0	0.01	0.11	0.03	0.01
	I. calycinum (GUA)			0.02	0	0.07	0	0	0	0	0	0	0	0	0	0
	I. gesnerioides (PUL)				0.26	0.09	0	0.01	0.19	0.23	0.31	0.24	0.25	0.31	0.28	0.26
C. ECU	***I. confertiflorum*** (MAL)					0.39	0	0.01	0.19	0.19	0.43	0.41	0.42	0.43	0.30	0.42
	I. fuchsioides (COJ)						0	0.01	0.01	0.08	0.09	0	0.01	0.09	0.03	0.01
S. ECU	***I. cyaneum*** (CIS)							0.72	0	0	0	0.17	0.17	0	0.17	0.17
	I. loxense (LOJ)								0.01	0.01	0.01	0	0.01	0.01	0.01	0.01
N. PER	***I. edule*** (AGA)									0.76	0.20	0.19	0.22	0.22	0.19	0.22
	I. parvifolium (AGA)										0.25	0.17	0.18	0.27	0.20	0.18
	I. umbellatum (AGA)											0.48	0.44	0.88	0.31	0.53
	I. ayabacense (AYA)												0.96	0.44	0.28	0.48
	I. lehmannii (AYA)													0.44	0.31	0.70
	I. peruvianum (GUZ)														0.31	0.46
	I. cornifolium (GUZ)															0.55
	I. stenanthum (GUZ)															

Figure 2. Pairwise similiarity in pollinator assemblage across *Iochroma* species. The names of the principally hummingbird-pollinated taxa are in boldface. Study site names are abbreviated as follows: AGA = Agallpampa, AYA = Ayabaca, CIS = Cisne, COJ = Cojitambo, GUZ = Guzmango, LOJ = Loja, MAL = Malpote, and PUL = Pululahua. Regions are abbreviated as follows: N. ECU = northern Ecuador, C. ECU = central Ecuador, S. ECU = southern Ecuador, and N. PER = northern Peru. Values for taxa in the same region are boxed in thin black lines, and taxa in sympatry are boxed in thick black lines. Shading of cells denotes degree of similarity.

significant preference for a particular species, although, in some cases, for the less rewarding species. For instance, over the course of three bouts, *A. melanogenys* visited 49 flowers on the low-reward *Iochroma umbellatum* and no flowers on *I. edule* ($P < 0.001$). Insect visits were typically too infrequent to show any significant pattern, but often they tended toward the less rewarding species (Table 4).

We also considered the movement of individual pollinators between these sympatric plant pairs. Although pollinator species could be observed visiting multiple sympatric plant species, we rarely saw movement between species by individual pollinators (Fig. 3). For instance, when observing an individual of *Iochroma cyaneum* and *I. lehmannii* side by side, only one of 60 visits involved movement from *I. lehmannii* to *I. cyaneum* and five involved movement in the reverse direction. The smaller hummingbirds (mentioned above) were largely responsible for these occasional interspecific movements. However, the overall rarity of interspecific movements between these individual plant pairs points to some individual-level pollinator fidelity. Due to the sparse distribution of *Iochroma* plants, we could not explore the frequency with which individual pollinators continued to be constant to a particular species after moving beyond the observed pairs.

POLLEN DEPOSITION AND POLLINATOR IMPORTANCE

Results from the bagged and unbagged controls suggested that the study species require biotic pollination. Styles from bagged flowers typically had very few pollen grains, but in three cases (*Iochroma calycinum*, *I. cornifolium*, and *I. peruvianum*) they had larger loads, with means of 134, 152, and 142 grains, respectively (Table 3). These loads, which are small relative to those typically on visited flowers, could be due to very small insects penetrating the mesh, but were more likely due to self-pollen deposited during anthesis. If the observed loads in bagged flowers of *I. cornifolium*, *I. peruvianum*, and *I. calycinum* are indeed self-pollen, they might not result in fertilization as crossing studies indicate that most *Iochroma* species are self-incompatible (Smith & Baum, 2007).

Comparison of the night- and day-bagged flowers indicated that, on average, 5.5 times more pollen was deposited during the day (0600–1900 hr.) than during the night (1900–0600 hr.; Table 3). In three species, *Iochroma ellipticum*, *I. umbellatum*, and *I. cornifolium*, pollen tube counts for the night-exposed flowers were greater than 50% that of day-exposed flowers. *Iochroma ellipticum* received the most visits during the

Table 4. Visitation patterns to sympatric species in Guzmango, Ayabaca, and Agallpampa, Peru. Pairs of plants of sympatric taxa were observed in the study localities (see Appendix 1), with interplant distances (d) in meters and the time observed in hours (hr.). For pairs of plants greater than 3 m apart, only hummingbird movements could be recorded. Species names are abbreviated to the first letters of the specific epithet. Although the larger study of *Iochroma cyaneum* involved an allopatric population, it was found in sympatry with other taxa in Ayabaca, Peru, and so was included in these observations. Observed plant and flower visits were compared to expected visits using a χ^2 analysis with Yates' correction. Expected values (in parentheses) are the number of visits expected if visits were directly proportional to display size. For instance, if one of the two plants being observed has twice as many flowers as the other, it would be expected to receive twice as many visits. Significant χ^2 values are noted with asterisks where * is $P < 0.05$ and ** is $P < 0.01$. Boldface values are significant after Bonferroni correction.

Site: Guzmango, Peru		Hummingbird visitors						Insect visitors					
		Coeligena iris			*Adelomyia melanogenys*			*Apis mellifera*			Vespid wasp		
Plant pair observed; time observed, interplant distance		*per*	*cor*	χ^2	*per*	*cor*	χ^2	*per*	*cor*	χ^2	*per*	*cor*	χ^2
I. peruvianum with 120 flowers and *I. cornifolium*	Plant visits	0 (3)	4 (1)	6.5*	3 (4)	3 (2)	0.4	7 (5)	0 (2)	1.7	2 (1)	0 (1)	0.02
with 50 flowers; 3.6 hr., 1.5 m	Flower visits	0 (19)	27 (8)	**61.4****	18 (36)	33 (15)	**28.9****	14 (10)	0 (4)	4.5*	2 (1)	0 (1)	0.02
I. peruvianum with 75 flowers and *I. cornifolium*	Plant visits				1 (1)	0 (0)		0 (1)	1 (0)	0.3	3 (2)	0 (1)	0.1
with 25 flowers; 4 hr., 1 m	Flower visits				2 (2)	0 (0)	0	0 (2)	2 (0)	2.7	5 (4)	0 (1)	0.6

Site: Ayabaca, Peru		Hummingbird visitors						Insect visitors								
		Coeligena iris			*Adelomyia melanogenys*			*Bombus*			cf. *Plebeia*			Vespid wasp		
Plant pair observed; time observed, interplant distance		*leh*	*cya*	χ^2	*leh*	*cya*	χ^2	*leh*	*cya*	χ^2	*leh*	*cya*	χ^2	*leh*	*cya*	χ^2
I. lehmannii with 60 flowers and *I. cyaneum*	Plant visits	1 (6)	16 (11)	4.3*	2 (2)	5 (5)										
with 125 flowers; 3 hr., 1 m	Flower visits	2 (66)	203 (139)	**91.0****	45 (30)	46 (61)	**11.0****									
I. lehmannii with 144 flowers and *I. cyaneum*	Plant visits	0 (7)	9 (2)	**32.1****	20 (18)	2 (4)	0.9	1 (1)	0 (0)		2 (2)	0 (0)		3 (2)	0 (1)	0.02
with 35 flowers; 3 hr., 2 m	Flower visits	0 (29)	36 (7)	**143.0****	224 (184)	5 (45)	**42.8****	10 (8)	0 (2)	1.3	4 (3)	0 (1)	0.1	5 (4)	0 (1)	0.3

Site: Agallpampa, Peru		Hummingbird visitors												Insect visitors					
		Colibri coruscans			*Adelomyia melanogenys*			*Myrtis fanny*			*Polyonymus caroli*			*Bombus*			*Apis mellifera*		
Plant pair observed; time observed, interplant distance		*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2
I. parvifolium with 220 flowers and *I.*	Plant visits	0 (1)	2 (1)	0.3	2 (2)	2 (2)											2 (1)	0 (1)	0.9
edule with 300 flowers; 2 hr., 2.5 m	Flower visits	0 (7)	16 (9)	**19.5****	15 (21)	34 (28)	**13.6****										7 (3)	0 (4)	7.3**
I. parvifolium with 50 flowers and *I.*	Plant visits							2 (5)	10 (7)	2.9	7 (4)	2 (5)	2.6						
edule with 60 flowers; 4.75 hr., 5 m	Flower visits							9 (66)	136 (79)	**88.5****	125 (80)	50 (95)	**46.6****						
I. parvifolium with 175 flowers and *I.*	Plant visits	1 (6)	16 (11)	5.7**										1 (0)	0 (1)	0.1	6 (2)	0 (4)	7.8**
edule with 300 flowers; 5 hr., 2.5 m	Flower visits	34 (108)	258 (184)	**78.6****										2 (1)	0 (1)	1.3	26 (10)	0 (16)	**41.9****
		umb	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2
I. umbellatum with 160 flowers and *I.*	Plant visits	0 (6)	18 (12)	6.8**	3 (1)	0 (2)	3.8												
edule with 350 flowers; 2.3 hr., 9 m	Flower visits	0 (39)	125 (86)	**55.7****	49 (15)	0 (34)	**104****												

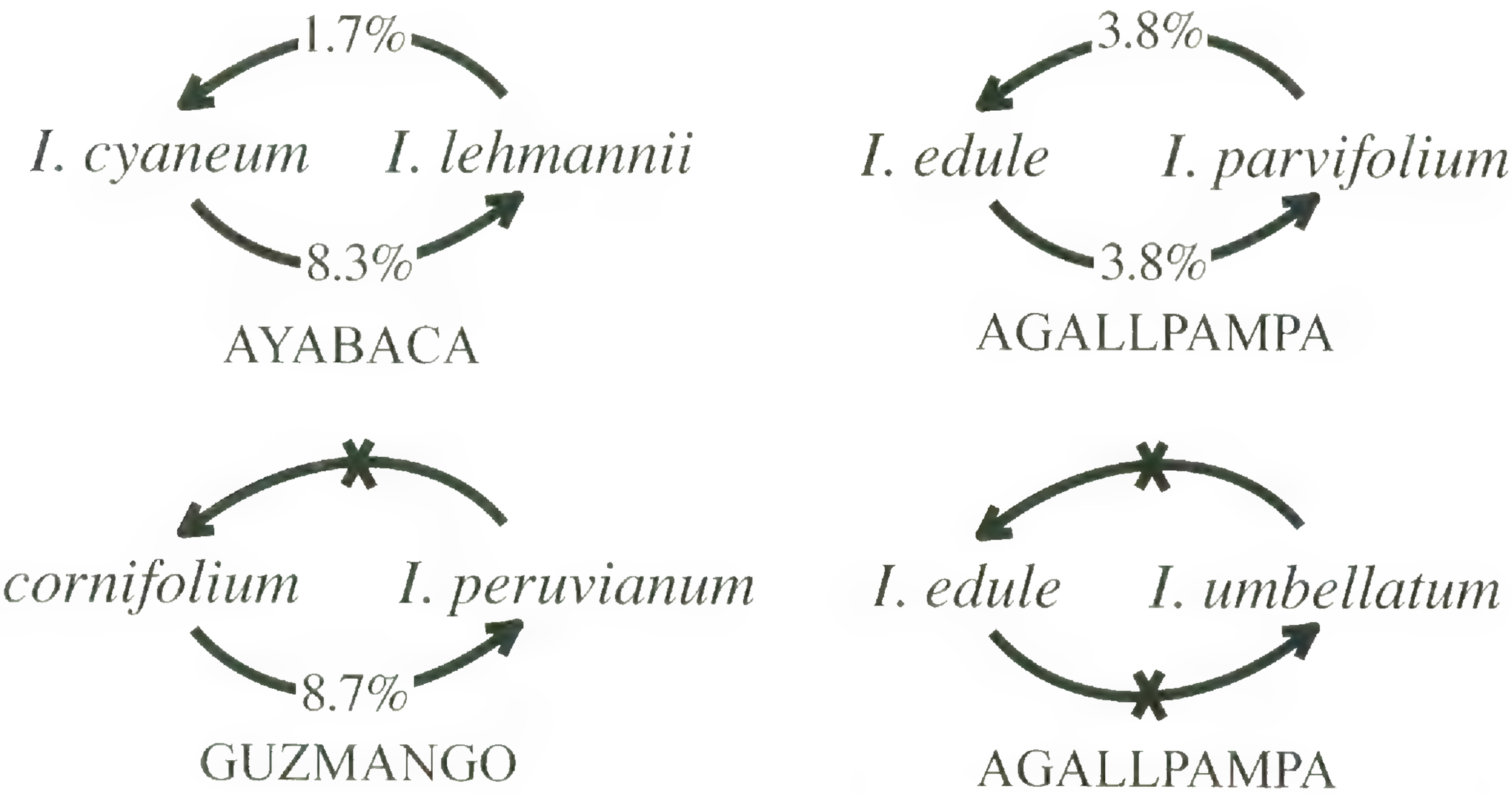

Figure 3. Observed interspecific pollinator movements among sympatric species in mixed populations. Arrows indicate percentage of interspecific movement observed between pairs of individual plants from different species, and crossed arrows indicate no observed interspecific pollinator movement. Sites where pairs were observed are given below pairs in capital letters. The percentage between *Iochroma peruvianum* and *I. cornifolium* is based on data from 23 visits to two pairs of plants, between *I. lehmannii* and *I. cyaneum* on 60 visits to two pairs of plants, *I. edule* and *I. parvifolium* on 52 visits to three pairs of plants, and *I. edule* and *I. umbellatum* on 21 visits on one pair of plants. Display sizes for these pairs are given in Table 1.

evening period (data not shown); thus, it was not surprising to find substantial night pollination in this species. Although no evening visits were observed for *I. umbellatum*, it could be visited by the same night-flying moths that visited the sympatric *I. edule* in the evening. It is unclear what could account for the night pollination in *I. cornifolium*, but the pollen loads (large relative to bagged flowers and comparable to visited flowers) implicate unidentified, nocturnally active animal visitors. Overall, however, the relatively small amounts of nocturnal pollen deposition in most taxa provide assurance that our largely diurnal pollinator observations covered most of the pollinator activity.

Thirty-four of the 47 pollinator species observed during the study visited virgin (previously bagged) flowers, allowing for estimation of pollen deposition. Visits by all species but one (an unidentified syrphid fly) resulted in pollen deposition (data not shown). Overall, hymenopterans deposited more pollen per visit on average than other classes of pollinators (Table 3). Dipteran visits resulted in the smallest deposition, on average (Table 3). Considering that the flowers have 50 to 500 ovules per flower (depending on species; Table 5), our deposition estimates suggest that single visits by most pollinator classes, except for dipterans, would result in enough pollen deposition to potentially fertilize all of the ovules. For instance, the average hummingbird visit deposited 264 viable pollen grains on a stigma of *Iochroma ayabacense* and, since this species has on average 124 ovules per flower, a single visit could potentially fertilize all the ovules.

Combining pollen deposition (quality) with visitation rate (quantity), we estimated the importance of each class of pollinators. For *Iochroma gesnerioides* and *I. umbellatum*, pollen counts were not available for the lepidopteran visitors. In the case of *I. gesnerioides*, we used the counts from its closest relative, *I. fuchsioides*, to estimate lepidopteran importance. For *I. umbellatum*, we used the average lepidopteran pollen deposition from *I. confertiflorum*, because for these two species, a hesperid butterfly species was the sole lepidopteran visitor. In this pollinator importance estimation, we scaled pollen deposition to equal the proportion of ovules potentially fertilized by a single visit. Since this proportion was 1.0 in most cases, pollinator importance values were similar to relative visitation (Table 5) as in Olsen (1997). Thus, hummingbirds appeared to be the most important pollinators for most *Iochroma* species, with only a few having either mixed bird-insect pollination or exclusively insect pollination (Table 5).

Discussion

Spatio-temporal considerations

Interactions between plants and their pollinators are subject to temporal variation within a day, within a season, and across years (Herrera, 1988; Schemske &

Table 5. Relative visitation rates and pollinator importance.

Species	Average ovules per flower ± SE	Scaled pollen deposition[1]				Relative flower visitation rates[2]				Relative pollinator importance[3]			
		Tro	Hym	Lep	Dip	Tro	Hym	Lep	Dip	Tro	Hym	Lep	Dip
Acnistus arborescens	52.0 ± 3.5	—	1	1	1	0	0.19	0.21	0.60	0	0.19	0.21	0.60
Iochroma ayabacense	123.5 ± 13.1	1	—	—	—	1.00	0	0	0	1.00	0	0	0
I. calycinum	488.0 ± 29.3	1	1	—	0.28	0.65	0.17	0	0.18	0.74	0.20	0.00	0.06
I. confertiflorum	152.3 ± 15.1	1	1	1	—	0.78	0.19	0.03	0	0.78	0.19	0.03	0
I. cornifolium	471.0 ± 22.6	1	1	—	—	0.96	0.04	0	0	0.96	0.04	0	0
I. cyaneum	368.7 ± 20.1	1	1	0.24	—	0.80	0.13	0.07	0	0.84	0.14	0.02	0
I. edule	414.7 ± 15.6	1	1	1	—	0.98	0.01	0.01	0	0.99	0.01	0.01	0
I. ellipticum	53.4 ± 13.0	—	1	1	1	0	0.38	0.38	0.23	0	0.38	0.38	0.23
I. fuchsioides	462.7 ± 7.6	0.60	1	0.69	0	0.77	0.16	0.06	0	0.70	0.24	0.07	0
I. gesnerioides	264.7 ± 19.8	1	1	1	—	0.90	0.06	0.04	0	0.90	0.06	0.04	0
I. lehmannii	69.3 ± 5.6	1	1	—	—	0.99	0.01	0	0	0.99	0.01	0	0
I. loxense	254.7 ± 21.7	1	1	—	—	0.89	0.11	0	0	0.89	0.11	0	0
I. parvifolium	69.1 ± 6.9	0.21	1	—	—	0.92	0.08	0	0	0.70	0.30	0	0
I. peruvianum	230.3 ± 11.6	1	1	0	0	0.44	0.56	0	0	0.44	0.56	0	0
I. stenanthum	146.0 ± 12.5	1	1	—	—	0.99	0.01	0	0	0.99	0.01	0	0
I. umbellatum	134.3 ± 7.2	1	1	1	0	0.52	0.47	4×10^{-3}	0.01	0.32	0.67	0.01	0

Tro = Trochilidae, Hym = Hymenoptera, Lep = Lepidoptera, and Dip = Diptera.

[1] Pollen deposition was scaled to equal the proportion of ovules potentially fertilized by a single visit.

[2] Relative visitation rates are the numbers of flowers visited per flower hour, rescaled to sum to 1.0.

[3] Relative pollinator importance is the product of the relative visitation and scaled deposition, rescaled to sum to 1.0.

Horvitz, 1989; Ivey et al., 2003; Price et al., 2005). Here, we sampled across the times of the day, but we did not sample in multiple years or times of the year and each study took place during a three- to five-day period. The issue of temporal variation is mitigated by the fact all studies took place in the same season (the rainy season), during which weather conditions did not vary substantially from day to day. Also, the pollinator fauna in the study areas are resident, as opposed to migratory (Greenewalt, 1960), and, thus, might be expected to shift less from year to year. Casual observations in the same locality across years (S. D. Smith, unpublished data) showed some variation in the animal species visiting a particular *Iochroma* species, but not in the broad class of pollinator species (e.g., bird or insect). Associations of plants with classes of pollinators are probably more robust across time than the specific composition of the pollinator assemblage.

Plant-pollinator associations may also vary across spatial scales, e.g., across sites (Boyd, 2004; Price et al., 2005) or along environmental gradients (Scobell & Scott, 2002; Herrera, 2005). Here, we have conducted studies of each species at a single site. Observations of several study taxa in other sites suggested that relative visitation of different classes of pollinators is similar across the species range despite variation in pollinator fauna (S. D. Smith, pers. obs.). For instance, in El Cisne, Ecuador, *Iochroma cyaneum* is mostly visited by the hummingbird *Amazilia amazilia* Lesson, whereas in Ayabaca, Peru, it is mainly visited by the hummingbirds *Coeligena iris* and *Adelomyia melanogenys*. Thus, while pollinator composition may vary across sites, *I. cyaneum* appears to be principally pollinated by hummingbirds across its range.

FLORAL DIVERSITY AND POLLINATOR RELATIONSHIPS

One goal in undertaking this study was to determine if the floral diversity in *Iochroma* corresponds to a diverse set of pollinator systems. At the broadest level, we observed three basic modes of pollination (bird, mixed bird/insect, and insect pollinated). Unlike other Andean taxa for which comparative pollination studies have been undertaken (Kay & Schemske, 2003; Pérez et al., 2006), pollination systems in *Iochroma* appeared only weakly related to floral differences (see also Smith et al., 2008). The insect-pollinated taxa *Acnistus arborescens* and *I. ellipticum* were both white, scented, and offered a low reward, but varied in shape and size (Fig. 1). The mixed bird-/insect-pollinated species *I. peruvianum* and *I. umbellatum* differed in flower color (one green, one orange), but shared two traits, small flowers and intermediate rewards (on a per-plant basis) (Table 1). The greatest floral variation was observed among the 12 bird-pollinated taxa, whose flower colors included red, white, yellow, blue, and purple and whose corolla size varied nearly three-fold (Fig. 1). One common feature among bird-pollinated species, however, was a large

nectar reward; all hummingbird-pollinated taxa had higher rewards than the mixed or exclusively insect-pollinated taxa (see also Smith et al., 2008). This observation is in accord with other studies showing that the amount of reward is more important than visual cues (e.g., flower color) in determining hummingbird visitation (Collias & Collias, 1968; Stiles, 1976; Melendez-Ackerman et al., 1997).

We also considered the possibility that the diversity of flower form among species sharing the same pollination system could reflect lower-level specialization, e.g., for particular pollinator species. However, we found no evidence to support such an explanation. A single hummingbird or insect species (e.g., *Adelomyia melanogenys* and *Apis mellifera* L.) was observed visiting multiple species of *Iochroma*, and, conversely, a single plant species was visited by multiple pollinator species (Appendix 2). For example, an average bird-pollinated *Iochroma* species was visited by 2.4 hummingbird species, and an average insect-pollinated species by 8.0 insect species. Furthermore, measurements of pollen deposition suggested that the vast majority of these visitors were effective pollinators. Thus, it appears that *Iochroma* species do not have tightly coevolved, specialized pollination systems.

Despite visits by many pollinator species, it is possible that a given *Iochroma* species could be specialized on a guild or functional group of pollinators, which collectively explain the particular floral traits seen. If this were the case, one might expect a lack of pollinator sharing among geographically proximate but florally distinct *Iochroma* species. However, our analysis of pollinator assemblage similarity showed that diverse taxa from the same geographic region and from the same study site shared pollinator species significantly more often than those from different regions or sites (Fig. 2). This is consistent with the idea that *Iochroma* species are generalists within a broad class (such as hummingbirds) and that they tend to be visited by whichever pollinator species are locally abundant.

POLLINATOR BEHAVIOR AND REPRODUCTIVE ISOLATION

The overlap in pollinator assemblage among sympatric taxa has significant implications for the maintenance of species boundaries. Considering that nearly all pollinators were capable of transferring loads of pollen in excess of the number of ovules on any given visit, any foraging bout that included visits to multiple species would almost certainly result in interspecific pollen flow. All sympatric species studied here shared at least two pollinator species, and some as many as four (Appendix 2), making interspecific pollen flow in sympatry potentially common. On the other hand, subtle differences in visitation rates and patterns appear to restrict interspecific pollen flow. First, PS values for sympatric taxa, even those with the same broad pollination system, were typically much lower than 1 (range, 0.20–0.96; mean, 0.51), reflecting differential visitation by pollinator species. Second, we observed that individual pollinators do not tend to move between sympatric species even when the plants are growing side by side (Fig. 3). This could be explained by individual preferences (Jones & Reithel, 2001) or optimal foraging (Heinrich, 1976; Waser, 1986), but is perhaps better explained by territoriality. Areas of *Iochroma* sympatry contained several hummingbird species, including small birds (e.g., *Adelomyia melanogenys* and *Polyonymus caroli*) and larger birds (e.g., *Colibri coruscans* and *Coeligena iris*). As mentioned previously, smaller hummingbirds tended to visit less nectar-rewarding species in mixed populations, even though there is no mechanical barrier preventing them from retrieving nectar from the more rewarding species. Larger, more aggressive birds dominate the more rewarding species and defend individuals of the rewarding species from the smaller birds (Feinsinger & Colwell, 1978; Stiles, 1981). One can envision that territorial behavior might prevent or reduce gene flow among plant species in mixed patches, and that this effect would be enhanced by differences in reward (Table 1). The combination of local spatial separation of populations, perhaps due to microhabitat specialization, and hummingbird territoriality might then reduce the potentially frequent interspecific pollen flow in sympatry. The low incidence of hybridization among sympatric *Iochroma* might reflect individual pollinator fidelity driven by interactions among hummingbirds, additional pre-fertilization mechanisms (e.g., pollen competition), and/or post-fertilization mechanisms. Although artificial interspecific crosses suggest wide crossability among *Iochroma* species (Smith & Baum, 2007; S. D. Smith, unpublished data), none of the species pairs that grow in sympatry without observed hybrids have yet been examined. Thus, additional crossing studies and tests of hybrid fitness will be required to understand how *Iochroma* species coexist in northern Andean communities.

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Appendix 1. Description of plant populations used in pollination studies in Ecuador and Peru.

Species	Voucher	Study dates	Plants studied	Avg. display per plant ± SE	Hours observed	Flower hours observed[1]
Acnistus arborescens (L.) Schltdl.	Ecuador, Alluriquin, 0.32°S 78.99°W, *S. D. Smith 209* (MO, QCNE, WIS)	26–28 Dec. 2002	8	21 ± 4	12.1	208.0
Iochroma ayabacense S. Leiva	Peru, Ayabaca, 4.63°S 79.71°W, *S. D. Smith & S. Leiva G. 337* (F, HAO, MO, NY, USM, WIS)	17–19 Jan. 2004	6	186 ± 45	14.9	3156.3
I. calycinum Benth.	Ecuador, Guajalito, 0.25°S 78.81°W, *S. D. Smith 471* (F, QCNE, WIS)	28–31 Dec. 2004	6	30 ± 11	18.7	784.6
I. confertiflorum (Miers) Hunz.	Ecuador, Malpote, 1.91°S 78.97°W, *S. D. Smith & L. Lopez 482* (MO, NY, QCNE, WIS)	16–18 Jan. 2005	6	112 ± 37	14.1	1770.8
I. cornifolium (Kunth) Miers	Peru, Guzmango, 7.39°S 78.90°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 337* (F, HAO, MO, NY, USM, WIS)	27–31 Jan. 2004	9	87 ± 16	23.9	2215.2
I. cyaneum (Lindl.) M. L. Green	Ecuador, Cisne, 3.86°S 79.43°W, *S. D. Smith 227* (MO, QCNE, WIS)	7–11 Jan. 2003	14	42 ± 6	36.6	1354.0
I. edule S. Leiva	Peru, Agallpampa, 7.95 S 78.56 W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 359* (F, HAO, MO, NY, USM, WIS)	6–8 Feb. 2004	6	311 ± 66	15.1	1773.2
I. ellipticum (Hook. f.) Hunz.	Ecuador, Los Gemelos, 0.63 S 90.38 W, *P. R. Izquierdo 15022* (CDRS)	8–10 May 2004	10	50[2]	12.0	200.0
I. fuchsioides (Humb. & Bonpl.) Miers	Ecuador, Cojitambo, 2.75°S 78.89°W, *S. D. Smith & L. Lopez 488* (F, NY, QCNE, WIS)	21–23 Jan. 2005	6	79 ± 14	21.1	1792.6
I. gesnerioides (Kunth) Miers	Ecuador, Pululahua, 0.039 N 78.50 W, *S. D. Smith 200* (MO, QCNE, WIS)	19–21 Dec. 2002	5	505 ± 253	12.0	7640.0
I. lehmannii Bitter[3]	Peru, Ayabaca, 4.63°S 79.71°W, *S. D. Smith & S. Leiva G. 330* (F, HAO, MO, NY, USM, WIS)	21–23 Jan. 2004	5	275 ± 119	13.2	3916.7
I. loxense (Kunth) Miers	Ecuador, Loja, 3.98 S 79.23 W, *S. D. Smith 499* (QCNE, WIS)	26–28 Jan. 2005	8	120 ± 38	13.4	1822.1
I. parvifolium (Roem. & Schult.) D'Arcy	Peru, Agallpampa, 7.95 S 78.56 W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 303* (F, HAO, MO, NY, USM, WIS)	4–8 Feb. 2004	6	98 ± 38	15.1	1806.1
I. peruvianum (Dunal) J. F. Macbr.	Peru, Guzmango, 7.39 S 78.90 W, *S. D. Smith & S. J. Hall 353* (F, HAO, F, MO, NY, USM, WIS)	29–31 Jan. 2004	4	85 ± 18	11.7	830.0
I. stenanthum S. Leiva, Quipuscoa & N. W. Sawyer	Peru, Guzmango, 7.39°S 78.90°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 313* (F, HAO, MO, NY, USM, WIS)	29–31 Jan. 2004	5	209 ± 52	12.0	2511.0
I. umbellatum (Ruiz & Pav.) Hunz. ex D'Arcy	Peru, Agallpampa, 7.95°S 78.56°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 360* (F, HAO, MO, NY, USM, WIS)	6–8 Feb. 2004	4	81 ± 40	17.8	1556.8
Total					263.58	36337.24

[1] Flower hours were calculated from a series of observations (1 to n) as follows: $FH = (D_1 \times T_1) + (D_2 \times T_2) + \ldots (D_n \times T_n)$, where FH equals the number of flower hours, D is the display size (number of flowers on the plant), and T equals the number of hours the plant was observed.

[2] Variation in display size across the population was not recorded in *Iochroma ellipticum*.

[3] We treat *I. lehmannii* and the recently described *I. squamosum* S. Leiva & Quipuscoa as synonyms.

Appendix 2. Pollinator taxa and visitation rates. Animals are listed by order and identified to species when possible, and only legitimate pollinators (which made contact with the reproductive organs) are included. Each unidentified visitor is given a unique number within its taxonomic group, and these identifiers are shared across plant species. For example, both *Iochroma cornifolium* and *I. peruvianum* were visited by Vespidae, sp. indet. 1. Only pollinators observed during the individual species studies are included; additional visitors observed during subsequent experiments (e.g., *Myrtis fanny*; Table 5) are not listed here. For each pollinator, plant and flower visitation rates (per flower hour) are listed (separated by a slash).

Acnistus arborescens	Hymenoptera	Apidae, *Apis mellifera* L., 0.014/0.048
		sp. indet. 1, 0.010/0.034
	Lepidoptera	sp. indet. 2, 0.010/0.091
	Diptera	Syrphidae, sp. indet. 1, 0.024/0.058
		Tipulidae, sp. indet. 1, 0.043/0.115
		sp. indet. 1, 0.019/0.048
		sp. indet. 2, 0.005/0.019
		sp. indet. 3, 0.005/0.014
Iochroma ayabacense	Apodiformes	Trochilidae, *Adelomyia melanogenys* Fraser, 0.005/0.089
		Trochilidae, *Coeligena iris* Gould, 0.005/0.097
I. calycinum	Apodiformes	Trochilidae, *Coeligena torquata* Boissoneau, 0.001/0.013
		Trochilidae, *Phaethornis* Swainson, 0.001/0.045
		Trochilidae, sp. indet. 1, 0.005/0.033
	Hymenoptera	Apidae, *Parapartamona vittigera* Moure, 0.001/0.001
		Apidae, *Plebia* sp. 2, 0.017/0.023
	Diptera	Drosophilidae, sp. indet. 1, 0.025/0.025
I. confertiflorum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.007/0.090
		Trochilidae, *Heliangelus viola* Gould, 0.004/0.082
	Hymenoptera	Apidae, *Apis mellifera*, 0.002/0.005
		Apidae, *Parapartamona vittigera*, 0.014/0.028
		Halictidae, *Caenohalictus* sp. indet. 1, 0.008/0.010
	Lepidoptera	Hesperidae, sp. indet. 1, 0.002/0.006
I. cornifolium	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.004/0.051
		Trochilidae, *Coeligena iris*, 0.008/0.125
	Hymenoptera	Apidae, *Apis mellifera*, 0.003/0.006
		Vespidae, sp. indet. 1, 0.001/0.001
I. cyaneum	Apodiformes	Trochilidae, *Amazilia amazilia* Lesson, 0.032/0.188
		Trochilidae, *Coeligena iris*, 0.008/0.049
	Hymenoptera	Vespidae, *Polybia* sp. indet., 0.027/0.040
	Lepidoptera	Sphingidae, sp. indet. 1, 0.001/0.007
		sp. indet. 1, 0.007/0.014
I. edule	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.002/0.033
		Trochilidae, *Colibri coruscans* Gould, 0.003/0.031
		Trochilidae, *Polyonymus caroli* Bourcier, 0.005/0.089
	Hymenoptera	Apidae, *Apis mellifera*, 0.0004/0.001
	Lepidoptera	Noctuidae, sp. indet. 1, 0.0003/0.001
I. ellipticum	Hymenoptera	Formicidae, *Paratrechina*, 0.002/0.002
		Formicidae, *Wasmannia auropunctata* Roger, 0.002/0.002
		Vespidae, *Pachodynerus galapagoensis* Williams, 0.003/0.005
	Lepidoptera	Geometridae, *Oxydia lignata* Warren, 0.002/0.002
		Noctuidae, *Agrotisia williamsi* Schaus, 0.002/0.002
		Noctuidae, sp. indet. 2, 0.002/0.003
		sp. indet. 3, 0.002/0.002
	Diptera	Syrphidae, *Xanthandrus agonis* Walker, 0.005/0.005
I. fuchsioides	Apodiformes	Trochilidae, *Coeligena torquata*, 0.001/0.021
		Trochilidae, *Heliangelus viola* Gould, 0.008/0.214
	Hymenoptera	Apidae, *Apis mellifera*, 0.004/0.027
		Halictidae, *Caenohalictus* sp. 2, 0.016/0.022
	Lepidoptera	Danaidae, sp. indet. 1, 0.003/0.020

Appendix 2. Continued.

I. gesnerioides	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.002/0.040
		Trochilidae, *Coeligena torquata*, 0.0001/0.004
		Trochilidae, *Boissonneaua flavescens* Loddiges, 0.004/0.102
		Trochilidae, sp. indet. 3, 0.0001/0.003
	Hymenoptera	Apidae, *Apis mellifera*, 0.001/0.010
	Lepidoptera	Sphingidae, sp. indet. 2, 0.001/0.006
I. lehmannii	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.002/0.148
		Trochilidae, *Coeligena iris*, 0.005/0.190
	Hymenoptera	Apidae, *Apis mellifera*, 0.001/0.003
		Apidae, *Plebeia*, 0.0003/0.001
I. loxense	Apodiformes	Trochilidae, *Amazilia amazilia*, 0.017/0.251
	Hymenoptera	Apidae, *Apis mellifera*, 0.001/0.002
		Colletidae, *Chilicola* cf. *pedunculata*, 0.005/0.005
		Vespidae, *Polybia*, 0.016/0.024
I. parvifolium	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.004/0.057
		Trochilidae, *Colibri coruscans*, 0.001/0.001
		Trochilidae, *Polyonymus caroli*, 0.010/0.230
		Trochilidae, sp. indet. 2, 0.001/0.021
	Hymenoptera	Apidae, *Apis mellifera*, 0.007/0.027
		Vespidae, sp. indet. 2, 0.001/0.001
I. peruvianum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.008/0.047
	Hymenoptera	Apidae, *Apis mellifera*, 0.021/0.055
		Vespidae, sp. indet. 1, 0.004/0.005
I. stenanthum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.006/0.192
		Trochilidae, *Coeligena iris*, 0.004/0.068
	Hymenoptera	Apidae, *Apis mellifera*, 0.002/0.004
I. umbellatum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.007/0.153
		Trochilidae, *Colibri coruscans*, 0.001/0.001
	Hymenoptera	Apidae, *Apis mellifera*, 0.019/0.131
		Anthophoridae, *Melissodes*, 0.003/0.009
	Lepidoptera	Hesperidae, sp. indet. 2, 0.001/0.001
	Diptera	Syrphidae, sp. indet. 2, 0.002/0.003

A SYNOPSIS OF THE GENUS *TACHIGALI* (LEGUMINOSAE: CAESALPINIOIDEAE) IN NORTHERN SOUTH AMERICA[1]

Henk van der Werff[2]

ABSTRACT

A synoptic treatment of the *Tachigali* Aubl., including *Sclerolobium* Vogel, in northern South America (Colombia, Venezuela, Guyana, French Guiana, Suriname, Amazonian Brazil, Ecuador, and Peru) is presented and contains brief descriptions of, and a key to, the 54 species recognized in northern South America. Ten species, *T. barnebyi* van der Werff, *T. bicornuta* van der Werff, *T. candelabrum* van der Werff, *T. cenepensis* van der Werff, *T. chrysaloides* van der Werff, *T. ferruginea* van der Werff, *T. fusca* van der Werff, *T. inconspicua* van der Werff, *T. loretensis* van der Werff, and *T. vaupesiana* van der Werff, are newly described. Three overlooked names published by Tulasne (*T. glauca*, *T. poeppigiana*, and *T. richardiana*) have been taken up again. One new combination, *T. melanocarpa* (Ducke) van der Werff, is proposed. Lectotypes are designated for three species, *T. alba* Ducke, *T. melanocarpa*, and *T. plumbea* Ducke. The following species are placed in synonymy: *Sclerolobium froesii* Pires in *T. bracteosa* (Harms) Zarucchi & Pipoly; *T. tessmannii* Harms in *T. formicarum* Harms; *T. myrmecophila* (Ducke) Ducke in *T. glauca*; *S. radlkoferi* Rusby, *S. uleanum* Harms, and *S. subbullatum* Ducke in *T. guianensis* (Benth.) Zarucchi & Herend.; *T. grandiflora* Huber, *T. ulei* Harms, *T. rusbyi* Harms, and *T. pulchra* Dwyer in *T. paniculata* Aubl.; *T. sulcata* Benoist and *T. bracteolata* Dwyer in *T. richardiana*; and *T. reticulosa* (Dwyer) Zarucchi & Herend. in *T. tinctoria* (Benth.) Zarucchi & Herend.

Key words: Northern South America, *Sclerolobium*, *Tachigali*, taxonomy.

TAXONOMIC HISTORY

The genus *Tachigali* was described by Aublet (1775), who recognized two species, *T. paniculata* Aubl. and *T. trigona* Aubl., both from French Guiana. The main difference between the two species given by Aublet was the position of the leaflets: opposite in *T. paniculata* and alternate in *T. trigona*. Jussieu (1789) introduced the orthographic variant "*Tachigalia*"; all later authors followed this until Lewis (1987) returned to the original spelling "*Tachigali*." Tulasne (1844) designated *T. paniculata* as the type species of the genus. Subsequently, the name *T. trigona* largely disappeared from use; Dwyer (1954) mentioned it as a synonym of *T. paniculata*, a disposition followed in this treatment. In 1837, Vogel published the genus *Sclerolobium*, with three species from central Brazil. Dwyer (1957a) designated *S. denudatum* Vogel as the type species. Bentham (1840) published a new species, *T. pubiflora* Benth., from Guyana. Tulasne (1844) treated *Tachigali* and included five species, four newly described, in the subgenus *Tachigali*, and two new species in his subgenus *Cosymbe* Tul. The subgenus *Tachigali* was characterized by dimorphic stamens, with three being thicker and backwards curved and seven longer and straight, and the ovary attached to the side of the hypanthium, while in subgenus *Cosymbe*, all 10 stamens are equal and the ovary is attached to the bottom of the hypanthium. Tulasne also treated two species in *Sclerolobium*, one of which, *S. sericeum* Tul., was newly described.

The names published by Tulasne have generally been ignored by later authors. Poeppig (1845) described two species, one in *Tachigali* and one in *Sclerolobium*, both of which had been described by Tulasne (1844) with the same collections as types. In a report on the plants collected by Spruce in Pará, Bentham (1850) gave a summary of *Sclerolobium*, in which he recognized nine species, three of which were newly described. In *Flora Brasiliensis*, Bentham (1870) listed 10 species of *Sclerolobium* and three species of *Tachigali*. Taubert, in Engler and Prantl (1892), accepted five species in *Tachigali* and 12 species in *Sclerolobium*. Taubert divided *Sclerolobium* in two sections, section *Eusclerolobium* (10 species) with linear petals and section *Platypetalum* (two species) with more or less broad petals. One of the two species in section *Platypetalum*, *S. aureum* (Tul.)

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Baill., had been placed by Tulasne (1844) in subgenus *Cosymbe* of *Tachigali*. Rusby (1896) described a new species of *Sclerolobium* from Bolivia.

From about 1900 onward, a sizable number of new species were described by two botanists working in Brazil, Huber and Ducke. Huber (1908) first described two new species of *Tachigali* followed by three new species of *Sclerolobium* (Huber, 1910). Ducke (1915, 1922, 1935, 1938, 1944) described nine species of *Sclerolobium* and six species of *Tachigali*. Ducke was an excellent collector, knew the species well from his field experience, and contributed greatly to our knowledge of *Tachigali*. His observations on habitat preference of the various species are very valuable. For most species described by Ducke, he used his own collections as type specimens.

In the same period, Harms (1903, 1906, 1915, 1922, 1926, 1940) described five species of *Sclerolobium* and five species of *Tachigali*. Harms studied collections made by other botanists (Ule, Tessmann, Weberbauer, Rusby, Melinon) and had not seen the plants in the field. Several of the species described by him have been placed in synonymy by later authors. Benoist (1919) added a species of *Sclerolobium* and later a species of *Tachigali* (Benoist, 1925), both based on his own collections. Macbride (1943) described *S. rigidum* J. F. Macbr. based on a Klug collection from Peru. The next major contribution was made by Dwyer, who published a revision of *Tachigali* in 1954, including the descriptions of three new species and a new variety, followed by a revision of *Sclerolobium* (Dwyer, 1957a) with the descriptions of one new species and three new varieties from the area covered by this synopsis. A key to the largest group of *Sclerolobium* was published separately (Dwyer, 1957b). Dwyer (1958) published an additional species of *Tachigali*, *T. schultesiana* Dwyer, from Colombia. Since 1960, five species of *Sclerolobium* have been described: *S. froesii* Pires (Pires, 1960), *S. dwyeri* R. S. Cowan, *S. pimichinense* R. S. Cowan (Cowan, 1961), *S. micranthum* L. O. Williams (Williams, 1965), and *S. prancei* H. S. Irwin & Arroyo (Irwin & Arroyo, 1974). Only one species of *Tachigali* has been described since Dwyer's publications: *T. vasquezii* Pipoly (Pipoly, 1995). With the large number of species in *Sclerolobium* and *Tachigali*, it became increasingly clear that the differences between the two genera were tenuous, and there is now a general consensus that the two genera should be merged under the older name *Tachigali*. Several species of *Sclerolobium* were recently transferred to *Tachigali* or given new names in three floras and a checklist (Zarucchi & Herendeen, 1993; Pipoly, 1995; Barneby, 1996; Zarucchi, 1998).

Tachigali versus *Sclerolobium*

In the original publication of *Tachigali*, Aublet (1775) listed, among others, the following characters: stamens unequal, with three short, erect and pressed against the upper petals, and seven slender, resting on the lower petals; petals unguiculate; upper three petals reflexed, the lower two spreading; four sepals erect and one bent down. Vogel (1837) did not mention unequal stamens or differences in the orientation of the sepals and petals in his description of *Sclerolobium*; he did mention that the petals were thread-like, linear or nearly so ("fila angustissimo-linearia"). In his description of *Tachigali*, Vogel mentioned unequal petals, unequal stamens, and the attachment of the stipe of the ovary to the upper side of the hypanthium. The species on which these descriptions were based represent the extremes of the range of floral variation found in the *Tachigali–Sclerolobium* complex. Tulasne (1844) recognized two subgenera in *Tachigali*: subgenus *Tachigali*, characterized by unequal stamens and the pistil inserted along the upper wall of the hypanthium, and subgenus *Cosymbe*, with equal stamens and the pistil inserted at the bottom (or center) of the hypanthium. The latter subgenus was placed in *Sclerolobium* by later authors (as section *Platypetalum* [Taubert, 1892]) (Dwyer, 1957a). The place of attachment of the pistil was a character first used by Tulasne and became later correlated with the shape of the calyx cup: symmetrical when the pistil is inserted at the bottom of the hypanthium, oblique when the pistil is attached along the side of the hypanthium.

During the past two decades, several authors have indicated a close relationship between the two genera. In 1993, Gentry stated *Sclerolobium* "is probably not adequately distinct from *Tachigali*." Zarucchi and Herendeen (1993) merged *Sclerolobium* in *Tachigali*, as did Barneby and Heald in Mori et al. (2002), Pennington et al. (2004), Lewis in Lewis et al. (2005), and Silva and Lima (2007). Detailed reasons for merging *Sclerolobium* and *Tachigali* were, however, not presented in any of these publications. The present study finds that the species of *Tachigali* can be divided into five groups based on the characters used by Aublet, Vogel, and Tulasne (petals linear or broad; stamens equal or unequal; hypanthium symmetrical or asymmetrical; and sepals and petals erect or spreading/reflexed). These species groups are presented in Table 1. The first and largest group corresponds with *Sclerolobium* sensu Vogel; the second group with *Tachigali* subg. *Cosymbe* sensu Tulasne (= *Sclerolobium* sect. *Cosymbe* sensu Dwyer); the third group is close to subgenus *Cosymbe*, but differs in having flowers with spreading sepals and

Table 1. Species groups in *Tachigali*. Names in boldface refer to species that are not monocarpic (collections exist with leaves and fruits); italicized names refer to species with swollen domatia in the leaf stalk.

Group 1: petals linear, stamens equal, calyx symmetrical, sepals/petals erect		Group 2: petals broad, stamens equal, calyx symmetrical, sepals/petals erect	Group 3: petals broad, stamens equal, calyx symmetrical, sepals/petals spreading	Group 4: petals broad, stamens equal, calyx asymmetrical, sepals/petals spreading	Group 5: petals broad, stamens unequal, calyx asymmetrical, sepals/petals spreading
T. amplifolia	***T. macbridei***	*T. candelabrum*	*T. dwyeri*	T. alba	T. barnebyi
T. bracteosa	**T. melanocarpa**	**T. davidsei**	T. micrantha	*T. argyrophylla*	*T. catingae*
T. cenepensis	**T. melinonii**	T. ferruginea	**T. micropetala**	***T. glauca***	***T. cavipes***
T. chrysaloides	*T. odoratissima*	*T. formicarum*		*T. grandistipulata*	**T. colombiana**
T. chrysophylla	**T. paraensis**	**T. macropetala**		*T. loretensis*	T. longiflora
T. eriopetala	**T. peruviana**			*T. poeppigiana*	**T. macrostachya**
T. fusca	***T. physophora***			***T. schultesiana***	***T. paniculata***
T. goeldiana	T. pimichinensis				*T. plumbea*
T. guianensis	T. prancei				*T. ptychophysca*
T. hypoleuca	**T. setifera**				T. pubiflora
T. inconspicua	**T. tinctoria**				T. richardiana
T. leiocalyx	*T. vaupesiana*				*T. rigida*
	T. vulgaris				***T. venusta***

petals; the fourth group has previously been considered part of *Tachigali* sensu Aublet. None of the groups with *Tachigali* sensu Aublet. None of the groups differs in more than one character from its neighboring group(s). If *Sclerolobium* and *Tachigali* were both to be recognized at the generic level, they would be separated by a single character only. For instance, if *Sclerolobium* would be defined by linear petals, species in group 1 would be placed in *Sclerolobium* and those in groups 2, 3, 4, and 5 in *Tachigali*. However, if *Tachigali* would be defined by an asymmetrical hypanthium, species in groups 4 and 5 would form *Tachigali* and those in groups 1, 2, and 3 would be placed in *Sclerolobium*. Recognition of such single character genera is not recommended. Therefore, in this study, as in other recent treatments mentioned above, *Sclerolobium* is included in *Tachigali*.

Characters Useful in Identification

Flowers

Flowers are almost indispensable for the identification of *Tachigali* specimens. The indument of the calyx is rather uniform in most species, but a few have glabrous calyx lobes. The shape of the hypanthium, symmetrical or asymmetrical, is of more importance. In about half the species the petals are linear; the other half have broad, more or less showy petals. The linear petals can be difficult to recognize because they closely resemble the filaments of the stamens, but they can usually be told apart by a slight difference in color. The linear petals are densely pubescent on the distal half in a number of species, and the pubescence is usually of yellow hairs, but in one species, *T. paraensis* (Huber) Barneby, the hairs are white. There is also variation among the species with broad petals. In some species, the petals are erect, shorter than, or as long as the sepals and largely covered by them, while in others, the petals are spreading to reflexed and easily seen. This study did not find the amount of pubescence on the inner surface of the petals to be a useful character for discriminating species. The number of stamens is generally 10, with one exception: *T. macrostachya* Huber is described as having 15 (16 to 19) stamens. In the flowers seen for this treatment, the number of stamens is approximately 15, but because it is not uncommon for a few stamens to break off, the original number may well have been higher. Stamens may be equal or unequal in length; in the latter case, two or three stamens are shorter, thicker, and curved. The lower part of the filaments is nearly always densely pubescent. This study did not find the distribution or the density of

pubescence on the ovary to be of taxonomic significance; even in very young fruits the pubescence falls early.

STIPULES

Stipules have not been seen for 17 of the 54 species included in this treatment. In a number of species, they were difficult to find or rarely present. Three main types of stipules are found in *Tachigali*. In 23 species, stipules were pinnately divided, with one to three pairs of lobes; the lobes were flat and of varying width but never thread-like. This is the foliose stipule type (Figs. 1A, B, 4D). In nine species, the stipules were pinnately divided but with thread-like segments. This is the pectinate stipule type (Fig. 1C, D). The remaining six species had entire stipules (rarely with a single pair of lobes); these stipules were strongly revolute and subcylindrical in cross section (Fig. 1E, F). In some cases, small bracts resembling revolute stipules were present in the inflorescences. Such bracts occurred in flowering specimens of *T. eriopetala* (Ducke) L. F. Gomes da Silva & H. C. Lima, but in sterile specimens of that species, revolute stipules at the base of the leaves were up to 5 cm long. Because the foliose stipule type occurs in quite a few species, they are not very useful for identification. Revolute stipules, however, are quite useful; sterile specimens of *T. eriopetala*, for instance, can be easily identified by the combination of stipule type and indument type on the lower surface of the leaflets. Within a species, there may be some variation in the size of stipule but not in the type of stipule.

DOMATIA

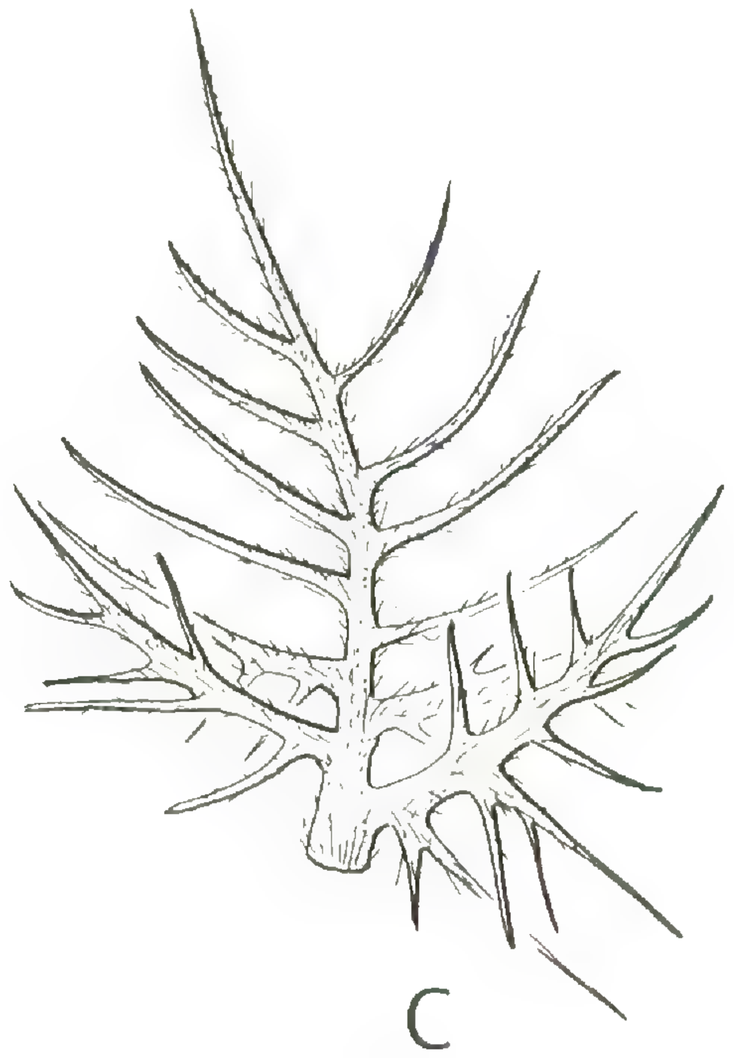
Domatia are swollen and hollow segments of the leaf stalk inhabited by ants; they are present in 26 species and absent in 29 species. Presence or absence of domatia seems to be characteristic for any given species, with the exception of *Tachigali paniculata*, where domatia can be present or absent. *Tachigali paniculata* has been included in both categories in the count. Shape and position of domatia sometimes aid identification. Domatia can be terete (Fig. 2A–C) or, when flat on the upper side of the leaf stalk, semiterete (Fig. 2D, E). In one species, *T. bicornuta* van der Werff, the domatia form two blunt horns (Fig. 4C). In *T. physophora* (Huber) Zarucchi & Herend., domatia are terete, often longitudinally ribbed, and almost never more than 1 cm from the base of the leaf stalk (Fig. 2A). In *T. chrysophylla* (Poepp.) Zarucchi & Herend., domatia are usually more than 4 cm from the base of the leaf stalk (sometimes even 10 cm) and can be up to 4 cm long (Fig. 2B). Table 1 lists species with domatia in italics and shows that the majority of species belonging to the traditional concept of *Tachigali* (with an asymmetrical hypanthium and spreading petals, and dimorphic or equal stamens) possess domatia. *Tachigali longiflora* Ducke and *T. macrostachya*, two species listed in this group as lacking domatia, have hollow leaf stalks that are inhabited by ants, but in these species the leaf stalks are not swollen. Presence of domatia is much less common among species belonging to the traditional concept of *Sclerolobium*.

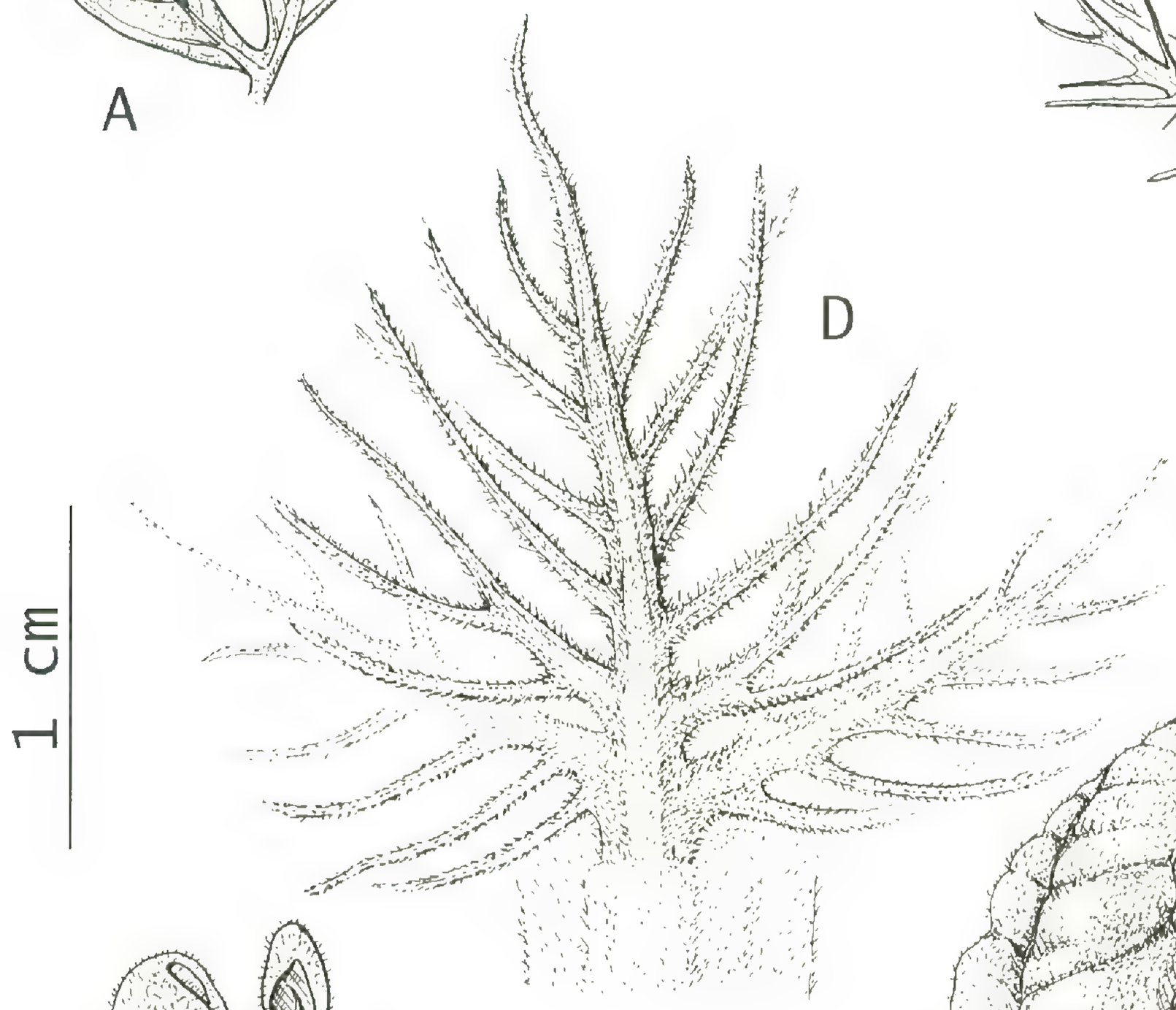
LEAFLETS

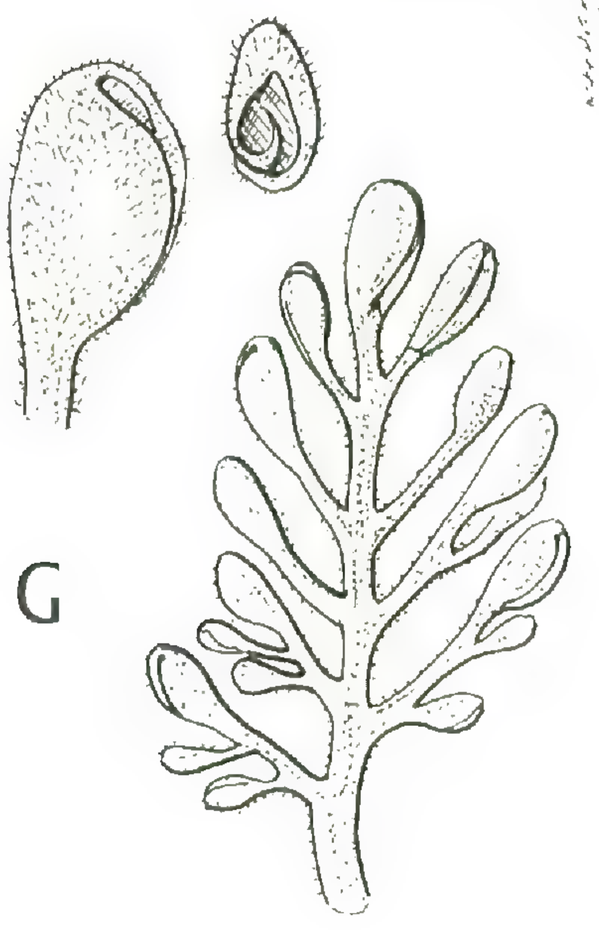
Leaflet characters such as size, number of lateral veins, pubescence, and shape of base and apex are of limited use in identification, and it is rarely possible to identify a specimen based only on leaflets. Characters that are uncommon in northern South American *Tachigali* such as leaflets with two to four pairs of secondary veins in *T. ptychophysca* Benth. and *T. loretensis* van der Werff, or a cordate base in *T. macrostachya*, merely serve to confirm a determination based on other characters.

FRUITS

Some species of *Tachigali* are reported to be monocarpic (Foster, 1977), but it is not known how many display monocarpy. In known monocarpic species, fruiting is the last phase of life after the leaves have fallen, and it is not possible to connect fruiting specimens with flowers or leaves of other collections. Fruits on their own cannot be identified due to a lack of diagnostic characters. Occasionally, field observations indicate that a species is monocarpic; for instance, O. Poncy (pers. comm.) collected flowering material of *T. richardiana* Tul. in a permanent plot and, on returning a year later, found that all the trees had died. In the case of *T. formicarum* Harms, another monocarpic species, a collection consisting of fruits and a leaf rachis could only be identified because of the characteristic shape of the domatia. Species that are not monocarpic (known from collections with fruits and leaves) are listed in boldface in Table 1 and are fairly equally distributed among the species groups. The species of which fruits are not yet known might be monocarpic and/or are possibly rare so that fruits remain uncollected. For instance, *T. catingae* Ducke is known only from the flowering type collection and two additional sterile collections; therefore, in this case it would be premature to consider this species as monocarpic.







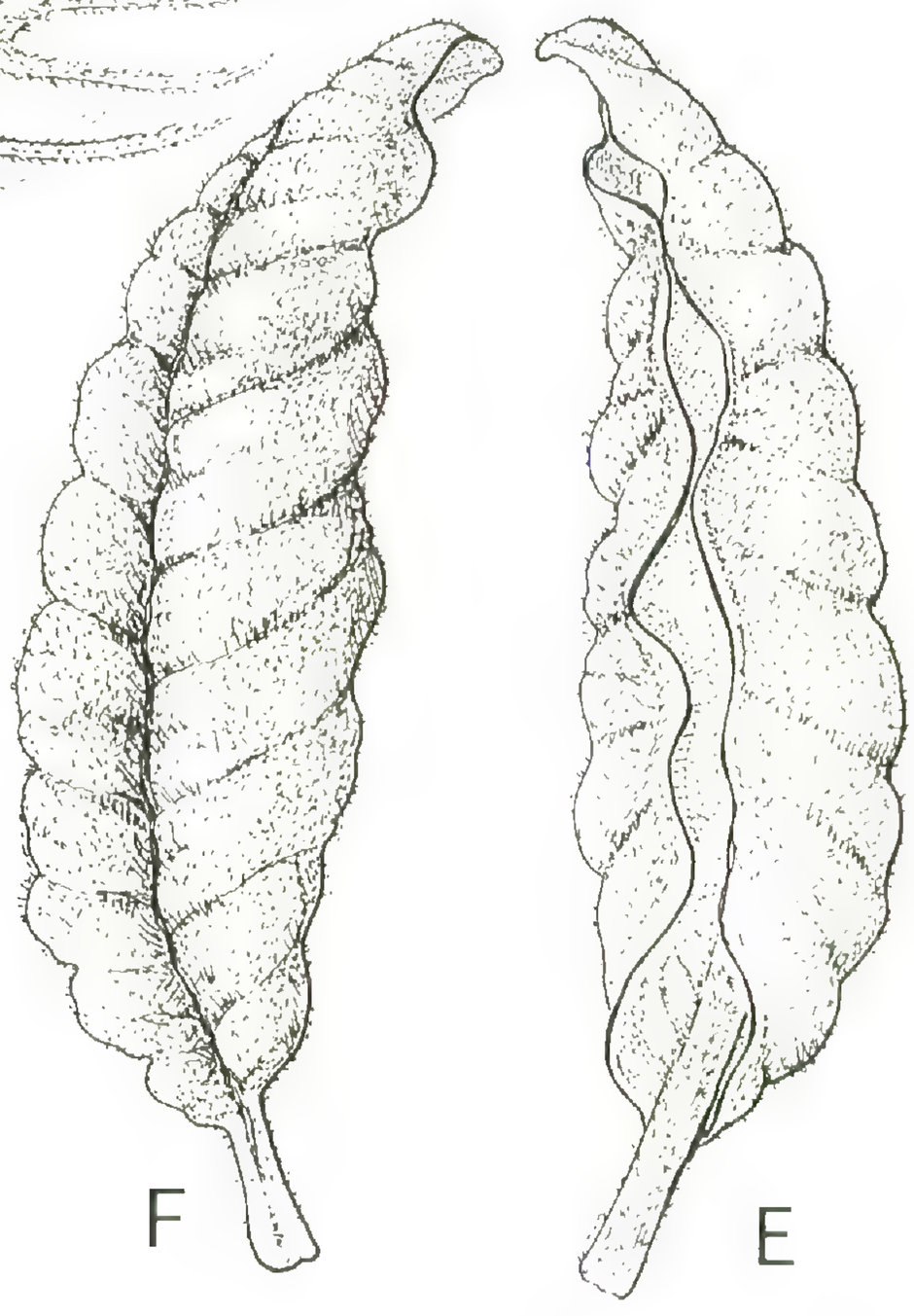


Figure 1. Stipules. —A. *Tachigali physophora* (*Huber 530*, US). —B. *T. cavipes* (*Pires 7920*, IAN). —C. *T. chrysophylla* (*Steyermark 107178*, US). —D. *T. ferruginea* (*Neill 12932*, MO). —E, F. *T. chrysaloides* (*Dorr & Barnett 5824*, NY). G. *T.* sp. 1 (*Rickson B-44A-85*, MO).

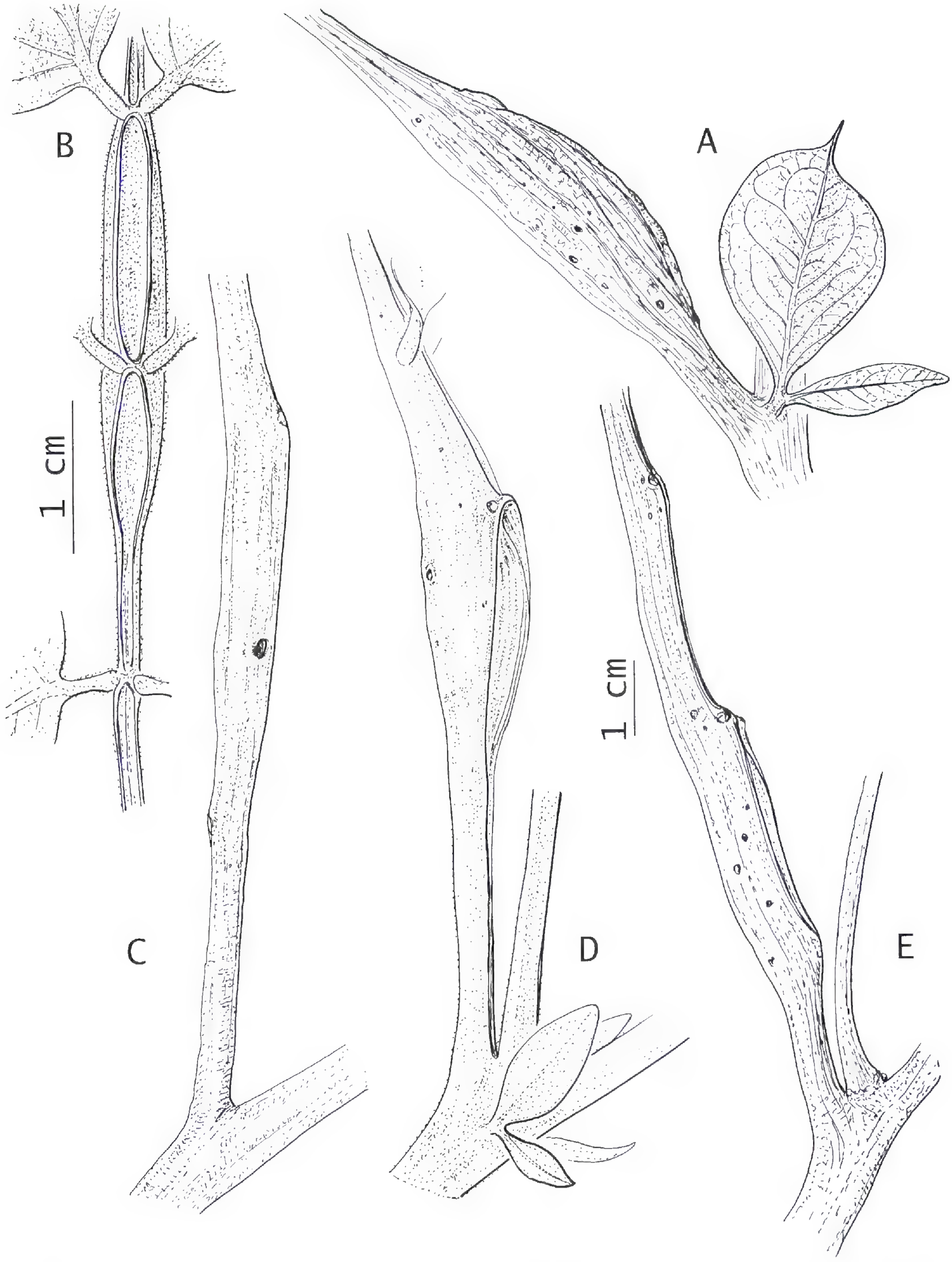


Figure 2. Domatia. —A. *Tachigali physophora* (*Huber 530*, US). —B. *T. chrysophylla* (*Steyermark 107178*, US). —C. *T. glauca* (*Prance et al. 24720*, US). —D. *T. cavipes* (*Pires 7920*, IAN). —E. *T. formicarum* (*Schunke 5255*, US). Scale bar at left applies to A–C; scale bar at right applies to D, E.

Scope of this Project

The treatment of *Tachigali* presented here was begun during preparation of an account for the *Flora del Río Cenepa* (Vásquez Martínez et al., in press). It soon became clear that identification of herbarium specimens was very difficult. Because large loans of specimens from an earlier attempt to study *Tachigali* were still at MO, it was decided to identify these collections as far as possible, write a key to the species, and combine this with brief notes in a synoptic treatment. This account only deals with the species from northern South America (Colombia, Venezuela, Guyana, French Guiana, Suriname, Amazonian Brazil [states of Amapá, Pará, Roraima, Amazonas, Acre], Ecuador, and Peru). The species in central and southern Brazil, Bolivia, and Paraguay are, for the most part, taxonomically different from those in northern South America, and, because not many collections from the southern area were at hand, this project was limited to species from northern South America. Identifications of the more than 1300 collections are not included in this treatment, but are available at: <http://www.mobot.org/MOBOT/Research/NorthernSouthAmerica/NorthernSouthAmerica.shtml>; click on the link to the Excel spreadsheet. This account is not a full monograph, and a number of problems remain to be studied in more detail, especially in the variable and widespread species *T. paniculata* and *T. guianensis* (Benth.) Zarucchi & Herend.

Taxonomic Treatment

Tachigali Aubl., Hist. Pl. Guiane 372. 1775. TYPE: *Tachigali paniculata* Aubl.

Sclerolobium Vogel, Linnaea 11: 395. 1837. TYPE: *Sclerolobium denudatum* Vogel.

Small to large trees, some species monocarpic. Leaves alternate, singly pinnate, paripinnate, leaflets opposite; leaf stalks sometimes swollen and harboring stinging ants; stipules present or caducous, pectinate, pinnate or entire, sometimes strongly revolute. Inflorescences terminal or lateral racemes or panicles; bracts lanceolate or subulate, often caducous. Flowers yellow, rarely white; sepals 5, equal or unequal, basally united, the hypanthium symmetrical or asymmetrical; petals 5, linear to broad, sometimes densely pubescent; stamens 10, rarely 15 to 16, equal or with 3 shorter, thicker and curved filaments usually densely pubescent on the basal half; ovary stipitate, attached in the center of the hypanthium or along the upper side, pubescent. Fruits strongly laterally compressed, dry, 1- to 2-seeded.

Distribution. A genus of 60 to 70 species from Costa Rica to southern Brazil and Paraguay.

Key to *Tachigali* Species in Northern South America

1a. Domatia in the leaf petioles with 2 blunt, horn-like projections, these ca. 1 cm long . . . 5. *T. bicornuta*
1b. Domatia absent or present; when present, cylindrical and never with horn-like projections 2
2a. Leaves with scattered, small (ca. 0.2 mm), stellate hairs . . . . . 33. *T. melinonii*
2b. Leaves without scattered, stellate hairs; indument, if present, of simple hairs 3
3a. Petals longer than sepals, more or less spreading, readily visible in open flowers; or, if petals about as long as sepals, then petals obvious, about as wide as the sepals 33
3b. Petals equal to or shorter than the sepals, inconspicuous, linear or nearly so, clearly much narrower than the sepals 4
4a. Leaf petiole or rachis with swollen, hollow, ant-inhabited domatia 5
4b. Leaf petiole or rachis without ant-inhabited domatia 13
5a. Sepals glabrous except the ciliate margin; twigs and inflorescences fuscous-tomentulose/tomentose . . . . . 19. *T. fusca*
5b. Sepals uniformly pubescent; indument of twigs and inflorescences variable, but never fuscous . . . 6
6a. Lower surface of the leaflets completely covered by the indument 7
6b. Lower surface of the leaflets partially covered by the indument or glabrous 11
7a. Leaflets 15–30 cm long, with 18 to 25 pairs of secondary veins; base of leaflets cordate, symmetrical . . . . . 17. *T. ferruginea*
7b. Leaflets to 20 cm long, with 6 to 15 pairs of secondary veins; base of leaflets obtuse to acute, symmetrical or oblique 8
8a. Indument of twigs and inflorescences consisting of erect hairs . . . . . 29. *T. macbridei*
8b. Indument of twigs and inflorescences consisting predominantly of appressed hairs, sometimes a few erect hairs present as well 9
9a. Base of leaflets asymmetrical; stipules pectinate or foliose, flat 10
9b. Base of leaflets symmetrical; stipules small, entire, strongly revolute . . . . . 52. *T. vaupesiana*
10a. Stipules pectinate; domatia more than 4 cm from base of leaf, 2–4 cm long; mostly trees of terra firme forest . . . . . 12. *T. chrysophylla*
10b. Stipules foliose, flat; domatia ca. 1 cm from base of leaf, 1–2 cm long; trees of flooded forest or river's edge . . . . . 10. *T. physophora*
11a. Inflorescences and leaf rachises glabrous or nearly so; base of leaflets asymmetrical; domatia usually longitudinally ridged . . . 40. *T. physophora*
11b. Inflorescences and leaf rachises densely puberulous; base of leaflets symmetrical; domatia smooth, not longitudinally ridged 12

12a. Leaflets to 4 cm wide; lower surface of leaflets glabrous or very sparsely appressed pubescent; trees of flooded forest . . . . . 36. *T. odoratissima*
12b. Leaflets 6–11 cm wide; lower surface of leaflets with scattered erect hairs; trees of non-flooded forest . . . . . 10. *T. cenepensis*
13a. Sepals glabrous, at least on the distal half 14
13b. Sepals uniformly and usually densely pubescent . . . 16
14a. Lower surface of leaflets rather densely pubescent, the hairs ferruginous, erect . . . . . 34. *T. micrantha*
14b. Lower surface of leaflets glabrous or nearly so . . . 15
15a. Leaflets oblong to oblong-obovate, the margin often recurved; sepals 1–1.5 mm long; petals about half as long as sepals, broad, glabrous . . . . . 35. *T. micropetala*
15b. Leaflets elliptic to elliptic-ovate, the margin flat; sepals 2–2.5 mm long; petals about as long as the sepals, filiform to narrowly spatulate, pubescent . . . . . 26. *T. leiocalyx*
16a. Lower surface of leaflets moderately to densely pubescent with whitish hairs, these seemingly radiating from a central point in various directions . . . . . 50. *T. setifera* s. str.
16b. Lower surface of leaflets without hairs radiating from a central point 17
17a. Lower surface of leaflets glabrous to moderately pubescent, the surface always partially or entirely visible 18
17b. Lower surface of leaflets completely covered by the indument, this varying from sericeous to tomentulose 26
18a. Indument on twigs, leaves, and inflorescences pilose, consisting of erect hairs, these sparse or moderately dense 19
18b. Indument, when present, appressed 20
19a. Base of leaflets asymmetrical; stipules pectinate, the segments flat, linear; petals linear, glabrous . . . . . 23. *T. guianensis*
19b. Base of leaflets symmetrical; stipules 1- to 3-foliose, the segments revolute; petals linear, distally densely yellow pubescent . . . . . 50. *T. setifera* s.l.
20a. Lower surface of leaflets with dark brown scurf or minute scales (ca. 0.1 mm), this varying from dense to sparse; individual hairs not visible . . . 50. *T. setifera* s.l.
20b. Lower surface of leaflets glabrous or variously pubescent, but never with dark brown scurf; if pubescent, individual hairs visible under a dissecting scope 21
21a. Petals pubescent; base of leaflets symmetrical or asymmetrical 22
21b. Petals glabrous; base of leaflets asymmetrical . . . 25
22a. Petals white pubescent . . . . . 38. *T. paraensis*
22b. Petals yellow pubescent 23
23a. Stipules caducous; small (to 5 mm), entire, stalked, strongly revolute bracts sometimes present in the inflorescences; base of leaflets cuneate . . . . . 32. *T. melanocarpa*
23b. Stipules foliose, sessile, often more than 1 cm long and wide, persistent; entire, strongly revolute bracts lacking; base of leaflets obtuse 24
24a. Base of leaflets symmetrical or slightly asymmetrical; secondary veins 8 to 11 pairs; young leaflets with some appressed hairs . . . . . 25. *T. inconspicua*
24b. Base of leaflets asymmetrical; secondary veins 5 to 7 pairs; young leaflets silky pubescent on the lower surface . . . . . 2. *T. amplifolia*
25a. Flowers pedicellate . . . . . 54. *T. vulgaris*
25b. Flowers sessile or nearly so . . . . . 51. *T. tinctoria*
26a. Indument on young twigs and leaf rachis predominantly erect 27
26b. Indument on young twigs and leaf rachis appressed 28
27a. Leaflets oblong to oblong-ovate, 2 to 3× longer than wide; petals pilose; floral bracts not longer than mature buds . . . . . 44. *T. prancei*
27b. Leaflets broadly elliptic to elliptic-obovate, up to 2× longer than wide, or if 2× as wide as long, then leaflets less than 10 cm long; petals glabrous; floral bracts much longer than mature buds . . . . . 6. *T. bracteosa*
28a. Tips of leaflets obtuse to rounded . . . . . 41. *T. pimichinensis*
28b. Tips of leaflets acute or acuminate 29
29a. Petals pilose 30
29b. Petals glabrous 32
30a. Stipules flat, 3-foliose or entire; trees from flooded forest . . . . . 24. *T. hypoleuca*
30b. Stipules strongly revolute, entire; trees from terra firme forest 31
31a. Leaflets elliptic; secondary veins 6 to 10 on each side; tertiary venation immersed, not visible . . . . . 16. *T. eriopetala*
31b. Leaflets oblong; secondary veins 14 to 20 on each side; tertiary venation raised, visible on the lower surface of the leaflets . . . . . 11. *T. chrysaloides*
32a. Base of leaflets symmetrical; floral bracts twice as long as the floral buds; flowers sessile . . . . . 21. *T. goeldiana*
32b. Base of leaflets asymmetrical; floral bracts caducous, not present at base of larger buds; flowers pedicellate . . . . . 39. *T. peruviana*
33a. Trees from the Pacific lowlands in Colombia . . . . . 13. *T. colombiana*
33b. Trees or shrubs east of the Andes 34
34a. Sepals with a fringe of hairs along the margin, otherwise glabrous . . . . . 30. *T. macropetala*
34b. Sepals uniformly and densely pubescent 35
35a. Indument on inflorescences and flower buds very dark brown; hypanthium in young fruits ca. 1 cm long . . . . . 27. *T. longiflora*
35b. Indument on inflorescences and flower buds light brown or grey-brown; hypanthium in young fruits less than 7 mm long, usually less than 5 mm long 36
36a. Secondary veins of each leaflet 2 to 3 pairs 37
36b. Secondary veins of each leaflet 5 or more pairs . . . 38
37a. Stamens dimorphic; petals 5–6 mm long; long stamens 10–12 mm long . . . . . 45. *T. ptychophysca*
37b. Stamens monomorphic; petals ca. 3 mm long; stamens ca. 5 mm long . . . . . 28. *T. loretensis*

38a. Lower surface of leaflets densely appressed pubescent to sericeous; sometimes erect hairs present as well 39
38b. Lower surface of leaflets sparsely pubescent or glabrous 16
39a. Domatia absent; hairs on inflorescences and leaf axes partly erect . . . . . 46. *T. pubiflora*
39b. Domatia present; hairs on inflorescences and leaf axes appressed or erect 40
40a. Hairs on inflorescences and leaf axes erect or ascending 41
40b. Hairs on inflorescences and leaf axes strictly appressed 42
41a. Stamens monomorphic, 4–6 mm long; stipules 2–5 cm long, to 3 cm wide, foliose, entire or with one basal lobe . . . . . 22. *T. grandistipulata*
41b. Stamens dimorphic, the longer ones 1–2 cm long; stipules often lacking, when present up to 2 × 0.5 cm . . . . . 48. *T. rigida*
42a. Stamens monomorphic; petals ca. 4 mm long; domatia terete in cross section 43
42b. Stamens dimorphic; petals 5–7 mm long; domatia semiterete in cross section 44
43a. Inner surface of the petals pubescent for at least 1/5 of their length; only known from the lower Rio Negro, Parana de Anavilhana . . 3. *T. argyrophylla*
43b. Inner surface of the petals with a few hairs near the base; mostly from Pará, few collections from Amapá, Roraima, and Guyana . . . . . 20. *T. glauca*
44a. Flowers sessile or nearly so, a distinct pedicel lacking; stipules with filiform segments . . . . . 53. *T. venusta*
44b. Flowers with a distinct pedicel, 3–4 mm long; segments of stipules narrowly to broadly ovate, widest in the middle 45
45a. Hypanthium distinctly asymmetrical; along Rio Vaupes and tributaries in Brazil and Colombia and around Iquitos, Peru . . . . . 9. *T. cavipes*
45b. Hypanthium slightly asymmetrical; lower Rio Negro, downstream from Rio Jauaperi . . . . . 12. *T. plumbea*
46a. Diameter of flowers ca. 5 mm; stamens monomorphic 47
46b. Diameter of flowers 10 mm or more (rarely only 8 mm); stamens dimorphic 53
47a. Leaves with 10 or more pairs of leaflets 48
47b. Leaves with up to 6 pairs of leaflets 50
48a. Inner surface of the petals densely pubescent with long hairs, the hairs extending beyond the margins of the petals; inflorescences ca. 30 cm, with longitudinal ridges due to the decurrent bases of the flowers . . . . . 7. *T. candelabrum*
48b. Inner surface of the petals pubescent, but hairs not extending beyond the margins; inflorescences terete, rarely exceeding 20 cm, not longitudinally ridged 49
49a. Hairs on calyx appressed, grey-brown; hypanthium asymmetrical . . . . . 43. *T. poeppigiana*
49b. Hairs on calyx minute, ascending to erect, medium brown; hypanthium symmetrical or nearly so . . . . . 19. *T. schultesiana*
50a. Domatia semiterete; petals densely yellow pubescent on the inner surface, the hairs long and extending beyond the margin of the petals . . . . . 18. *T. formicarum*
50b. Domatia cylindrical or lacking; petals sparsely pubescent on the inner surface, the hairs not extending beyond the margin of the petals 51
51a. Domatia lacking; inflorescences and flowers densely reddish brown pubescent . . . 14. *T. davidsei*
51b. Domatia present; inflorescences and flowers densely brown puberulous or moderately to sparsely pubescent 52
52a. Inflorescences and flower buds densely puberulous and completely covered by indument; inner surface of the petals pubescent, the hairs covering about 3/4 of the surface . . . . . 28. *T. loretensis*
52b. Inflorescences and flower buds moderately to sparsely pubescent, the surface always partially visible; inner surface of petals with a few hairs near the base, otherwise glabrous . . . . 15. *T. duckei*
53a. Base of leaflets cordate; margin of leaflets revolute; leaflets bullate; stamens 15 or more . . . . . 31. *T. macrostachya*
53b. Base of leaflets obtuse to cuneate; margin of leaflets not revolute; leaflets plane; stamens 10 54
54a. Leaves with 3 (rarely 4) pairs of leaflets; leaflets twice as long as wide; domatia present . . . . . 8. *T. catingae*
54b. Leaves with more than 5 pairs of leaflets; leaflets more than twice as long as wide; domatia present or absent 55
55a. Leaf rachis sharply triangular; domatia present or absent . . . . . 37. *T. paniculata*
55b. Leaf rachis terete or nearly so; domatia absent . . . 56
56a. Stamens monomorphic; secondary veins 8 to 10, often slightly impressed on the upper surface . . . . . 1. *T. alba*
56b. Stamens dimorphic; secondary veins 4 to 8, immersed on the upper surface 57
57a. Stipules caducous; lower surface of leaflets sparsely to moderately appressed pubescent, not gland-dotted . . . . . 47. *T. richardiana*
57b. Stipules persistent, foliose; lower surface of leaflets glabrous, densely gland-dotted . . . . . 4. *T. barnebyi*

1. **Tachigali alba** Ducke, Arch. Jard. Bot. Rio de Janeiro 3: 92. 1922. *Tachigali paniculata* var. *alba* (Ducke) Dwyer, Ann. Missouri Bot. Gard. 41: 240. 1954. TYPE: Brazil. Pará: "prope Gurupa," *Ducke s.n. (MG 17227)* (lectotype, designated here, MG not seen, photo F!; duplicates, BM!, G not seen, photo F!, P!).

Discussion. *Tachigali alba* is a tall (30–40 m) tree from terra firme forests. Domatia are lacking, stamens are monomorphic, stipules are caducous and have not been seen. Leaflets have an asymmetrical base, are generally oblong with slightly impressed lateral veins on the upper surface. The leaf rachis is terete or nearly so. Flowers are smaller than in *T. paniculata*, with petals 4–4.5 mm and sepals 4 mm. The ultimate

divisions of the inflorescences are rather loosely flowered and linear, while in the similar *T. paniculata* the flowers are more compactly clustered and the flower-bearing part is narrowly pyramidal, clearly wider at the base than at the apex. *Tachigali alba* is morphologically close to *T. richardiana*; differences are the dimorphic stamens in *T. richardiana*, fewer pairs of lateral veins (8 to 10 in *T. alba*, 4 to 7 in *T. richardiana*), and the presence of a patch of hairs near the base on the outer surface of the petals, while the outer surface of the petals is glabrous in *T. alba*.

To prevent nomenclatural ambiguity, the collection *Ducke s.n. (MG 17227)* in MG has been designated as lectotype. The other syntypes are *Ducke s.n. (MG 17075)* and *Ducke s.n. (MG 17110)*; duplicates of these collections have been seen in BM and represent *Tachigali alba*.

Distribution. Tachigali alba is known from Amapá, Amazonas, and Pará in Brazil and a single collection from Guyana.

Selected specimens examined. BRAZIL. **Amapá:** *Pires et al. 51242* (F, GH, IAN, MG, US). **Amazonas:** Manaos, *Ducke 932* (F, MG, R, RB, US). **Pará:** Rio Pracupi, *Froes 32856* (IAN, US). GUYANA. Rewa River, at Great Falls, *Clarke 6563* (K).

2. Tachigali amplifolia (Ducke) Barneby, Brittonia 48: 182. 1996. Basionym: *Sclerolobium amplifolium* Ducke, Arq. Inst. Biol. Veg. 2: 43. 1935. TYPE: Brazil. Amazonas: "prope São Paulo de Olivença, silva non inundabili," *A. Ducke s.n. (RB 24295)* (holotype, RB not seen; isotypes, K!, P!, US!).

Discussion. According to the original description, this species is recognized by its large leaflets that are densely pubescent; linear petals; large and persistent, foliose stipules; and lack of domatia. The description from Ducke (1935) also mentioned that the leaflets were silky pubescent and golden-shiny, but these characteristics are restricted to young leaflets. Petals are pubescent on the upper half. Leaflets have an asymmetrical base. Four collections have been seen from western Brazil (Acre, Amazonas, Rondônia) and several from Guyana, French Guiana, and Suriname. The collections from the Guianas have larger leaflets (to 25 × 11 cm) and longer floral bracts than the collections from western Brazil. When recorded on field labels, habitat is given as forest on terra firme. Good flowering material of *Tachigali amplifolia* has not been seen from Guyana, French Guiana, or Suriname in this study, and it is not certain if the specimens from the Guianas should be included in *T. amplifolia*. The flowers of the specimens from western Brazil have relatively long sepals in relation to the hypanthium, and the sepals are rather narrow and do not overlap.

Selected specimens examined. BRAZIL. **Acre:** Mun. Mancio Lima, *Cid Ferreira et al. 10026* (INPA, NY). **Amazonas:** São Paulo de Olivença, *Krukoff 8879* (F, MO, NY, US). **Rondônia:** 30 km E of Rondônia en route to Vilhena, *Maguire et al. 56799* (COL, F, NY, US). FRENCH GUIANA. Haut Camopi, *Sabatier 1546* (MO, NY). SURINAME. Wilhelmina Gebergte, *Irwin et al. 55597* (GH, NY).

3. Tachigali argyrophylla Ducke, Bol. Tecn. Inst. Agron. N. 2: 14. 1944. TYPE: Brazil. Amazonas: Parana de Anavilhana, *Ducke 936* (holotype, RB not seen; isotypes, INPA!, K!, MO!, R!, US!).

Discussion. Characteristic of the species is the combination of the sericeous lower surface of the leaflets, terete domatia, and rather small, broad petals (4 mm long) with a pubescent inner surface. The base of the leaflets is asymmetrical. Stamens are monomorphic. Stipules are unknown. This species is currently known from the type collection from the lower Rio Negro in terra firme forest and possibly a second collection from Loreto, Peru. It is surprising that more collections of this species do not exist, because the lower Rio Negro is relatively well collected. A collection from the Jenaro Herrera in Peru (*Spichiger 4686*) is tentatively placed here; it keys to *Tachigali argyrophylla* based on flower size, monomorphic stamens, indument on the inner surface of petals, terete domatia, and sericeous lower surface of leaflets. This is an unusual disjunct distribution. Dwyer (1954) listed *Ducke 936* and *937* as syntypes. This is an error, since Ducke only mentioned *Ducke 936* when he described this species.

4. Tachigali barnebyi van der Werff, sp. nov. TYPE: Brazil. Rondônia: basin of Rio Madeira, margin of Mutumparaná airstrip, 25 Nov. 1968, *G. T. Prance, W. A. Rodrigues, J. F. Ramos & L. G. Farias 8844* (holotype, INPA!; isotypes, COL!, F!, GH!, MG!, R!). Figure 3.

Tachigali richardianae Tul. similis, sed ab ea stipulis persistentibus, foliolis subtus glabris punctatisque et petalis extus glabris recedit.

Tree, to 25 m; twigs terete, minutely puberulous, glabrescent; stipules foliose, gland-dotted on the lower surface, with a large terminal lobe (1.5–2.5 × 1–1.5 cm) and 1 or 2 small (4–5 × 2–3 mm) lateral lobes, the lobes with a short (1–2 mm) stalk. Leaf rachis 7–13 cm, terete, minutely puberulous, domatia lacking; leaflets 4 to 5 pairs, oblong to ovate-oblong, 7–10 × 2–3 cm, glabrous, gland-dotted on the lower surface, the base obtuse, symmetrical or nearly so, the apex acute; secondary veins 7 to 8 pairs per leaflet, immersed and poorly visible. Inflorescences 10–20 cm, densely brown puberulous. Flower buds

Figure 3. *Tachigali barnebyi* (isotype).

densely pubescent; bracteoles 4–5 mm, 1/2 to 1/3 the length of mature buds; pedicels of open flowers ca. 5 mm, hypanthium oblique, sepals 5–7 × ca. 4 mm, puberulous on the outer surface, pubescent on the inner surface, spreading to reflexed; petals similar in size and shape to the sepals, glabrous on the outer surface and pubescent on the inner surface, spreading to reflexed; stamens 10, dimorphic, 7 longer stamens ca. 1.2 cm, 3 shorter, ca. 8 mm, all pubescent on the lower 4 mm; pistil ca. 1.2 cm, ovary ca. 5 mm, densely pubescent, the style ca. 7 mm, becoming sparsely pubescent toward the stigma; stigma entire or with 2 short lobes. Immature fruits ca. 1 cm, densely pubescent; mature fruits not seen.

Discussion. *Tachigali barnebyi* shares with *T. richardiana* terete petioles, lack of domatia, and large flowers with dimorphic stamens. The terete petioles are especially rare among the species with large (4–7 × ca. 4 mm) petals. The new species differs from *T. richardiana* in its persistent stipules (caducous in *T. richardiana*) and its glabrous, gland-dotted lower surface of the leaflets (sparsely to moderately appressed pubescent and without gland dots in *T. richardiana*). Additional but weaker differences are the following: floral pedicels are longer in *T. barnebyi* (5 vs. 3 mm); the base of the leaflets is more asymmetrical in *T. richardiana*; and the leaflets of *T. barnebyi* are more coriaceous and the venation is less visible than in *T. richardiana*. The weaker differences are best seen when specimens of both species are at hand. The distribution of the two species does not overlap: *T. barnebyi* is known only from Rondônia and *T. richardiana* from French Guiana and adjacent Pará in Brazil. *Tachigali alba* also has terete petioles, has showy petals, and lacks domatia. However, it has caducous stipules, smaller petals (4–4.5 mm), monomorphic stamens, and slightly impressed secondary veins. According to the label of the type collection, isotypes have also been deposited in NY, K, S, U, and P.

Etymology. It is a pleasure to name this species after the late Rupert Barneby (1911–2000), who was truly a scholar and a gentleman and contributed so much to our understanding of Neotropical Leguminosae.

IUCN Red List category. *Tachigali barnebyi* is known from three collections in a poorly known part of the Amazonian rainforest and is therefore listed as Data Deficient (DD) (IUCN, 2001).

Paratypes. BRAZIL. **Rondônia:** Rodovia RO-399, a 13 km de Vilhena, *M. G. Vieira, R. H. Petersen, B. W. Nelson, J. F. Ramos & C. D. A. Mota 910* (INPA, MG, MO, US); Porto Velho, Rio Jamari, *U. N. Maciel & C. S. Rosario 1722* (MG).

5. Tachigali bicornuta van der Werff, sp. nov. TYPE: Peru. Amazonas: Bagua Province, distr. Imaza, comunidad Aguaruna de Wanas, cerros Chinim, 800–850 m, 31 Aug. 1996, *C. Diaz, A. Peña, L. Tsamajain & M. Roca 8100* (holotype, HUT not seen; isotype, MO!). Figure 4.

A congeneris domatiis petiolaribus bicornutis et ramulis terminalibus infructescentiarum inflatis recedit.

Trees, 12 m; twigs terete or slightly angular, glabrous. Leaves ca. 50 cm, with 6 to 10 pairs of leaflets, rachis ± round, minutely puberulous, the hairs ca. 0.1 mm, erect, not visible with the unaided eye; a domatium present ca. 5 cm from the base of the leaf, this shaped as 2 blunt horns ca. 1 cm and with a round tip, a pair of small (ca. 6 × 3 cm) leaflets attached to the sinus between the 2 horns of the domatium; leaflets narrowly elliptic, 12–20 × 3.5–4.5 cm, the base obtuse, somewhat asymmetrical, the apex tapering to a sharp point, the lamina gland-dotted on the lower surface, glabrous, midrib and secondary veins minutely puberulous as on the rachis, secondary veins 8 to 11 pairs per leaflet, petiolules 3–5 mm; detached stipules ca. 3 cm long, with 3 pairs of lanceolate segments, these 1.5–2 cm × 2–5 mm. Flowers and inflorescences unknown. Detached infructescences ca. 25 cm, densely and minutely puberulous, 1- or 2-branched, base of infructescence solid, 3–6 mm diam., ultimate branches of infructescences 8–18 cm, distally swollen and hollow, ca. 1 cm diam., with 1 or 2 perforations near the base; calyx cup ± symmetrical, not strongly oblique; fruits strongly laterally compressed, ca. 5 × 2 cm, the margin papyraceous, 1-seeded.

Discussion. The new species *Tachigali bicornuta* can be easily recognized by the peculiar domatia on the leaf petioles, the finger-like inflated terminal branches of the infructescences, and the minute, erect indument on leaf rachis, the main veins of the leaflets, and the infructescences. According to the label information of the type collection, the petiolar domatia are occupied by fierce ants. A collection from Brazil, Acre, Serra do Moa (*Cid Ferreira et al. 5105*, INPA, MEL, MO, NY) has similarly inflated terminal branches of the inflorescences, which are 1–1.5 cm diam. This collection differs in several characters from the new species described here and is not placed in *T. bicornuta*. Its domatia are much longer than wide (ca. 5 × 1.5 cm) and are flattened on the upper surface of the rachis, much as in *T. formicarum*; its leaflets are glabrous and lack the minute puberulous indument on the main veins; the leaflets are larger and wider (up to 28 × 10 cm); and the indument on the inflorescences is appressed, not

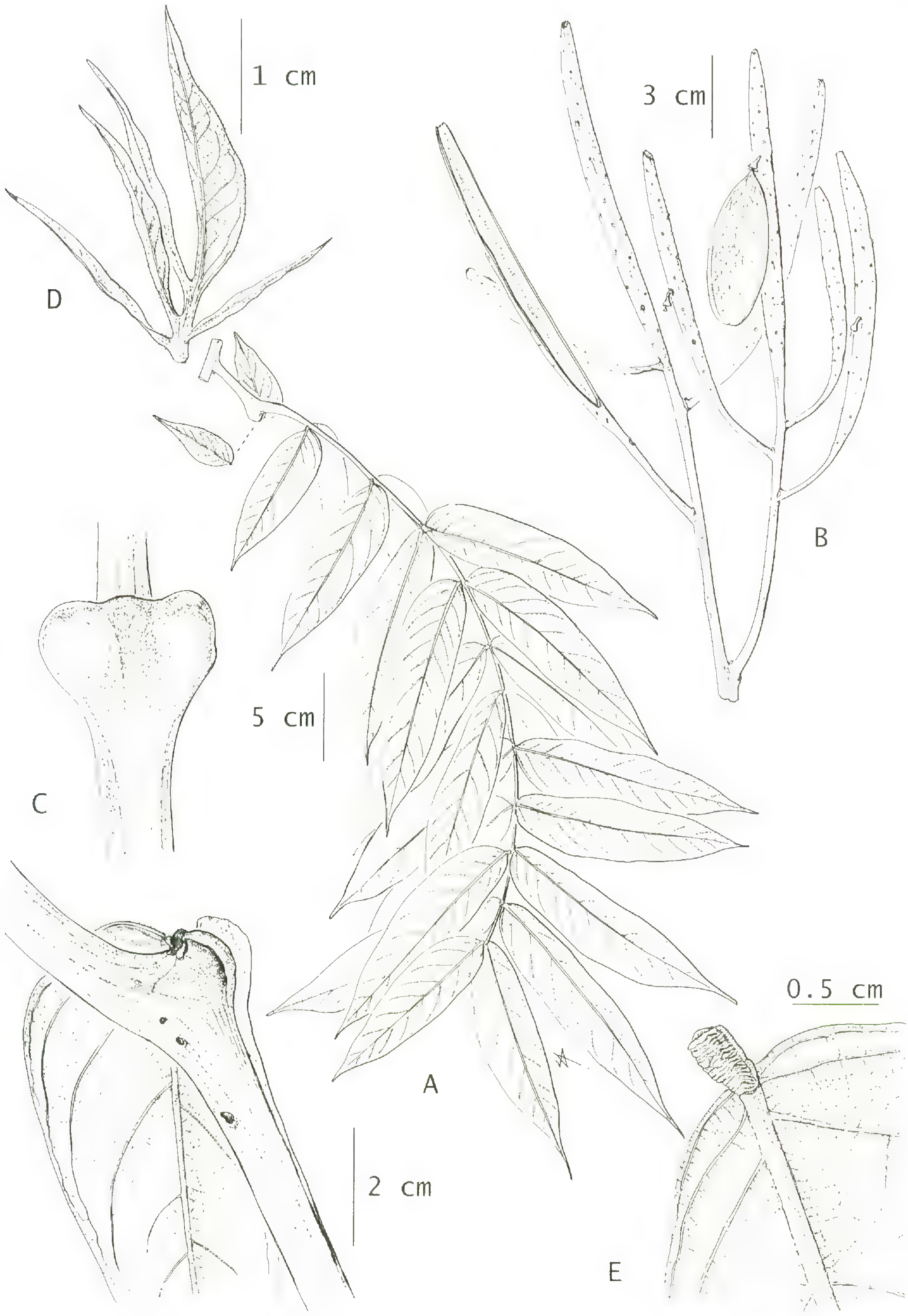


Figure 4. *Tachigali bicornuta* (*Diaz, Peña, Tsamajain & Roca 8100*, MO). —A. Leaf. —B. Infructescence. —C. Domatia, dorsal view (top) and lateral view (bottom). —D. Stipule. E. Base of leaflet.

puberulous or minutely erect. The hypanthium at the base of the mature fruits is symmetrical. Apart from the thickened terminal branches of the inflorescence, the collection agrees quite well with *T. formicarum*.

IUCN Red List category. *Tachigali bicornuta* has been collected once in a botanically poorly known region of Peru and is listed as Data Deficient (DD) (IUCN, 2001).

6. Tachigali bracteosa (Harms) Zarucchi & Pipoly, Sida 16: 411. 1995. Basionym: *Sclerolobium bracteosum* Harms, Bot. Jahrb. Syst. 40: 169. 1907. TYPE: Brazil. Amazonas: Cachoeiras des Marmellos, *Ule 6094* (holotype, B†; isotypes, K!, L!).

Sclerolobium froesii Pires, Bol. Tecn. Inst. Agron. N. 38: 23. 1960. *Tachigali froesii* (Pires) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 399. 2007. Syn. nov. TYPE: Brazil. Amazonas: Rio Canuma, *Froes 33744* (holotype, IAN not seen; isotype, RB not seen).

Discussion. *Tachigali bracteosa* has broadly elliptic to obovate leaflets, tomentulose on the lower surface, long bracts subtending the buds, and glabrous, linear petals. Domatia are lacking. Stipules of this species have not been seen in this study. The base of the leaflets is rounded to cordate. Only a photograph of the type of *Sclerolobium froesii* (in Pires, 1960) has been seen. The photograph and description leave no doubt that *S. froesii* is a synonym of *T. bracteosa*. Dwyer (1957a) cited *Macedo 4063* (MO, US) from the state of Pará under *S. bracteosum*; however, these specimens lack the tomentulose indument on the lower surface of the leaflets. In shape and size, the leaflets agree with *T. bracteosa*. It is possible that these fruiting specimens represent an undescribed species closely related to *T. bracteosa*. The US specimen of *Macedo 4063* has pectinate stipules.

Distribution. *Tachigali bracteosa* has been reported from flooded forest or riverbanks in Brazil (Amazonas, Mato Grosso, Tocantins) and Bolivia.

Selected specimens examined. BOLIVIA. **Beni:** Prov. Yacuma, *Beck & de Michel 20919* (NY). **Santa Cruz:** Parque Noel Kampff, *Quevedo et al. 2585* (NY). BRAZIL. **Amazonas:** Mun. Axinim, *Zarucchi et al. 2922* (INPA, MG). **Mato Grosso:** 1/2 km N of Rio Suia–Missu Ferry, *Ratter et al. 1383* (IAN, NY). **Tocantins:** Mun. Lagoa da Confusão, *Mendonça et al. 3962* (MO).

7. Tachigali candelabrum van der Werff, sp. nov. TYPE: Brazil. Amazonas: Mun. de Manicore, Rod. Transamazonica Km 302, Rio Aripuena entre a Rod. e a Cachoeira Matamata, 24 Apr. 1985, *Cid Ferreira 5780* (holotype, INPA! [2 sheets]; isotypes, MEL!, MG!, MO!, NY!). Figures 5 and 6.

Tachigali poeppigianae Tul. similis, sed ab ea lobis stipularum linearibus, petalis intus dense longeque pubescentibus, inflorescentiis majoribus, racemis porcatis et domatiis semiteretibus recedit.

Trees, to 12 m; twigs terete or slightly angled, smooth, glabrous; stipules 1–1.5 cm, pinnate, with 3 pairs of linear segments, these ca. 1 mm wide, glabrous or with a few scattered hairs. Leaves 25–40 cm, with 10 to 15 pairs or leaflets, the leaf axis sharply angled, glabrous or with some appressed hairs, leaflets in middle of the leaves 10–15 × 3–4 cm, often conduplicate, basal leaflets smaller, ca. 5 × 2.5 cm, leaflets glabrous, the base strongly asymmetrical, the larger lobe auriculate, the tip acute; secondary veins 6 to 8 pairs on each leaflet, inconspicuous, tertiary venation raised on the lower surface, immersed on the upper surface; domatia 2–3 cm, semiterete, not strongly thickened, distally with a pair of scars from fallen leaflets. Inflorescences paniculate, 30–40 cm, sparsely appressed pubescent near the base, the terminal racemes 20–25 cm, densely appressed pubescent and with pronounced ridges from the decurrent bases of the flowers; bracteoles ca. 5 mm, densely pubescent, linear; flowers yellow, densely and minutely appressed pubescent; pedicels 1–2 mm; sepals 2–3 mm, ovate-elliptic, densely pubescent on the outer surface, sparsely pubescent on the inner surface; petals slightly longer and narrower than the sepals, glabrous on the outer surface and pubescent on the inner surface, sometimes densely so toward the tip; stamens ca. 10, monomorphic, united at the base, 4–5 mm, pubescent on the lower half, ovary ca. 3 mm, densely and uniformly pubescent, the stalk ca. 1 mm; hypanthium asymmetrical, but ovary inserted at the base of the cup, with the stipe appressed against the lower side. Fruits not known.

Discussion. *Tachigali candelabrum* can be recognized by the leaves with many pairs of leaflets; the sharply angled leaf rachises; the dense pubescence on the inner surface of the broad petals, especially on the distal half; and the many ridges on the terminal racemes formed by the decurrent bases of the flowers. The long, upward-arching racemes give the impression of a candelabrum, and the chosen epithet "candelabrum" is a noun in apposition. *Tachigali poeppigiana* and *T. schultesiana* are the only other species of *Tachigali* with 10 or more pairs of leaflets. They differ in having terete domatia and foliose stipules and stamens, which are only pubescent near their base. *Tachigali candelabrum* is only known from three collections in southern

Figure 5. *Tachigali candelabrum* (holotype, leaf).

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Figure 6. *Tachigali candelabrum* (holotype, inflorescence).

Amazonas, east of Humaitá. One collection came from terra firme forest, one from igapo, and the third had no habitat information on the collection label.

The holotype consists of two sheets, one with a leaf, the other with an inflorescence. Both sheets have the same INPA herbarium number and form a single specimen.

IUCN Red List category. *Tachigali candelabrum* is only known from three collections. Without further information, it is best listed as Data Deficient (DD) (IUCN, 2001).

Paratypes. BRAZIL. **Amazonas:** Mun. de Humaitá, Rod. Transamazonica a 113 km de Humaitá, fl., *Cid Ferreira 5487* (INPA, MEL, MO, NY); 300 km E of Humaitá along Transamazonica Hwy., fl., *Cid Ferreira 5925* (MO, NY).

8. Tachigali catingae Ducke, Arq. Inst. Biol. Veg. 4: 12. 1938. TYPE: Brazil. Amazonas: *Ducke s.n. (RB 35421)* (holotype, RB not seen; isotypes, K!, P!, US!).

Discussion. *Tachigali catingae* can be recognized by the combination of glabrous leaves and twigs, while the inflorescences are rufous-brown tomentose or tomentulose; its leaflets are 3 to 4 pairs and 5–10 cm wide, stamens are dimorphic. Domatia are present, semiterete, and stipules are foliose with 1 pair of lateral lobes; the terminal lobe is ca. 2 cm long. Petals are elliptic to obovate, ca. 2 mm wide. The distal leaflets have a symmetrical, obtuse base. This species has relatively broad leaflets. The lower surface of the leaflets is densely gland-dotted, a character otherwise only seen in *T. barnebyi* and *T. bicornuta*.

Distribution. *Tachigali catingae* is known only from the type collection from the upper Rio Curicuriary and two sterile collections from the upper Rio Negro and the Rio Uaupes. As the name indicates, it occurs in caatinga forest.

Additional specimens examined. BRAZIL. **Amazonas:** Taracua, Rio Uaupes, *Pires et al. 7512* (IAN); Upper Rio Negro, Taracua, *Rodrigues 1141* (INPA).

9. Tachigali cavipes (Benth.) J. F. MacBr., Field Mus. Nat. Hist., Bot. Ser., 13: 127. 1943. *Tachigali paniculata* var. *cavipes* Benth., Fl. Bras. 15: 229. 1870. TYPE: Brazil. "prope Panure ad Rio Uaupes," *Spruce 2553* (holotype, K!; isotype, P!).

Discussion. This species is very similar to *Tachigali plumbea* Ducke; the only difference is the distinctly asymmetrical hypanthium in *T. cavipes* versus the slightly asymmetrical hypanthium in *T. plumbea*. The distributions of the two species do not overlap: *T. cavipes* is known from the Rio Vaupes and tributaries in Brazil and Colombia, one collection from the Río Guinía in Venezuela and a few collections around Iquitos in Peru, while *T. plumbea* occurs downstream along the Rio Negro and Rio Amazon. Petals are to 3 mm wide. Both species have semiterete domatia, sericeous leaflets, foliose stipules, and dimorphic stamens. *Tachigali glauca* Tul. and *T. venusta* Dwyer are also similar; *T. glauca* differs in its more erect or ascending indument on inflorescences and flowers and its monomorphic stamens, and *T. venusta* differs in its pectinate stipules and sessile flowers.

Selected specimens examined. BRAZIL. **Amazonas:** Rio Tiquié, affluent of Rio Uaupes, *Kawasaki 225* (INPA, MO). COLOMBIA. **Amazonas:** Rio Miritiparana, *Schultes & Cabrera 16463* (GH, US). **Vaupes:** Rio Piraparana, *Davis 119* (GH). PERU. **Loreto:** Jenaro Herrera, *van der Werff & Vasquez 9989* (MO). VENEZUELA. **Amazonas:** Rio Guainía, *Maguire et al. 41769* (F, GH, IAN, MO, US).

10. Tachigali cenepensis van der Werff, sp. nov. TYPE: Peru. Amazonas: "Quebrada chichijam entsa," 1100 ft., 24 Apr. 1973, *Ernesto Ancuash 291* (holotype, MO!; isotype, US!). Figure 7.

Quoad domatia longa *Tachigali chrysophyllae* (Poepp.) Zarucchi & Herend. similis, sed ab ea indumento ramulorum inflorescentiarumque erecto et basi foliolorum symmetrica differt.

Tree, 20–25 m; twigs angular, 1–1.5 cm thick, densely pubescent with short, erect, straight, ferruginous hairs; stipules foliose, flat, 2- or 3-lobed, the terminal lobe the largest, 1–1.5 cm long and wide. Leaf rachis 30–40 cm, densely ferruginous puberulous, with hairs similar to those on the twigs, domatia present, 8–10 cm, cylindrical, 7–9 mm diam., 8–10 cm from the base of the rachis and above the first pair of leaflets; leaflets ca. 5 pairs, the basal pair ca. 10 × 6 cm, the distal pairs larger, to 19 × 11 cm, the base symmetrical or nearly so, obtuse to rounded or subcordate, apices not seen (damaged on the type), sparsely pubescent with short, erect hairs on the lower surface, sometimes mixed with some longer, appressed hairs, glabrous on the upper surface; secondary veins 8 to 12 pairs per leaflet, immersed on the upper surface, raised and pubescent on the lower surface. Inflorescences paniculate, 20–30 cm, densely ferruginous puberulous, the main axes with cylindrical domatia similar to those of the leaf rachises, but shorter (5–8 cm long); ultimate branches of the inflorescences not swollen. Mature flowers densely pubescent, the pedicels ca. 1 mm long, the hypanthium cup-shaped, symmetrical, ca. 2 × 3 mm, sepals 2 2.5 × ca. 1.5 mm, petals as long as the sepals, linear, distally densely yellow pubescent; stamens 4–5 mm, pubescent near the base, otherwise glabrous;

Figure 7. *Tachigali cenepensis* (holotype).

young fruits ca. 8 mm, pubescent with spreading, dark brown hairs; floral bracts not seen. Mature fruits not seen.

Discussion. *Tachigali cenepensis* is a very distinct species based on the combination of long, cylindrical domatia; the dense, ferruginous indument consisting of short, erect and straight hairs on twigs and inflorescences; and the sparse, erect indument on the lower surface of the leaflets. The large leaflets with an obtuse, rounded, or subcordate base are also distinct. The species keys out together with *T. odoratissima* (Benth.) Zarucchi & Herend. and *T. physophora* because of the presence of domatia, uniformly pubescent sepals, and a sparse indument on the leaflets. However, these two species are not closely related to *T. cenepensis*. They differ in having smaller, narrower leaflets, smaller domatia, and a different indument; both are species from flooded forests in Venezuela, Brazil, and Colombia. Rather long, cylindrical domatia occur in *T. chrysophylla* and *T. macbridei* Zarucchi & Herend., but these two species have glabrous petals. It is more likely that the relationship of *T. cenepensis* rests with a group of species including *T. setifera* (Ducke) Zarucchi & Herend., *T. vasquezii*, *T. chrysaloides* van der Werff, and *T. prancei* (H. S. Irwin & Arroyo) L. F. Gomes da Silva & H. C. Lima. These species share oblong leaflets with a symmetrical base, pubescent petals, and, when present, large foliose or revolute stipules. *Tachigali cenepensis* is the only species in this group with domatia. Among the sterile or fruiting collections at MO are several collections from lowland Peru that closely resemble *T. cenepensis* in size and shape of leaflets and the dense, erect indument on the twigs and leaf stalks as well as one flowering collection resembling *T. cenepensis* in leaflet characters, but with appressed indument on the lower surface of the leaflets. Other collections from the same area, the valley of the Río Palcazu in Pasco, have short, erect hairs on the lower surface of the leaflets. Domatia are absent in all these specimens. It is possible that the domatia in the type specimens of *T. cenepensis* are atypical and that the specimens without domatia, but with similar leaflets, belong to *T. cenepensis*. The specimens similar to the type of *T. cenepensis* but without domatia differ from *T. setifera* s.l. in their larger and wider leaflets and leaves, thicker leaf stalks, and the dense, ferruginous indument on twigs and leaf stalks. Additional flowering specimens are necessary in order to resolve relationships in this group. The specimens resembling *T. cenepensis* are included in the exsiccatae list as *T.* aff. *cenepensis*; they had been previously identified as *T. vasquezii*, *Sclerolobium bracteosum*, and *S. rugosum* Benth. and may have been distributed under those names.

IUCN Red List category. This species is only known from the type collection. For lack of additional information, it is listed as Data Deficient (DD) (IUCN, 2001).

11. Tachigali chrysaloides van der Werff, sp. nov. TYPE: Ecuador, Morona-Santiago: Cordillera de Cutucu, along Patuca–Santiago rd., 600–1000 m, 23 Oct. 1988, *L. Dorr & L. Barnett 5824* (holotype, NY!; isotypes, MEL!, MO!). Figure 8.

Tachigali prancei (H. S. Irwin & Arroyo) L. F. Gomes da Silva & H. C. Lima similis, sed ab ea stipulis magnis revolutis et indumento breviore adpresso recedit; a *T. setifera* indumento sericeo et stipulis magnis diversa est.

Trees, to 30 m; twigs angular, minutely appressed pubescent; stipules entire or with one basal lobe, the main lobe or entire stipule 2–4 cm, strongly revolute, the lateral lobe when present 1–1.5 cm, also strongly revolute, appressed pubescent on the outer surface, densely so on the inner surface; the secondary veins strongly impressed and the whole structure appearing like a pupa of a butterfly. Leaf rachis 20–40 cm, terete or canaliculate on the upper surface, densely and minutely appressed pubescent, domatia lacking, leaflets 5 to 7 pairs, 11–20 × 4.5–8 cm, oblong to oblong-elliptic, the base symmetrical, rounded to subcordate, the apex acute or short-acuminate, the upper surface glabrous except for appressed pubescence on the primary and secondary veins, lower surface densely yellow-brown pubescent, the indument appressed and completely covering the surface, somewhat sericeous; secondary veins 14 to 20 pairs per leaflet, immersed on the upper surface, raised on the lower surface; tertiary veins scalariform, weakly raised on the lower surface. Inflorescences paniculate, 20–30 cm, the main axis densely appressed pubescent, the minor axes also with longer, curly hairs; pedicels 0.5–1 mm, floral bracts subulate, ca. 2 mm, densely pubescent, only present at base of young buds and quickly caducous; flowers 4–5 mm, uniformly appressed pubescent, the hypanthium ca. 1 mm long, turbinate, symmetrical, sepals ca. 3 mm, erect, the inner surface glabrous; petals linear, ca. 2 mm, densely yellow pubescent; stamens ca. 4 mm, pubescent on the lower half, distally glabrous; ovary ca. 3 mm, pubescent, the hairs stiff, brown, ascending. Fruit samaroid, ca. 15 × 3.5 cm, 1-seeded.

Discussion. *Tachigali chrysaloides* is closely related to *T. prancei* and *T. setifera*. It differs from both species by the large, revolute stipules. Stipules are generally absent in *T. prancei*; the one stipule seen

Tachigali chrysaloides van der Werff
Holotype
Determined by: Henk van der Werff, 2007

Figure 8. *Tachigali chrysaloides* (holotype).

in this study is foliose and flat. In *T. setifera*, stipules are also frequently absent; when present, they are to 1 cm long and revolute, as in *T. chrysaloides*. The indument on twigs and leaf rachises of *T. prancei* consists of short, erect hairs and is not appressed as in *T. chrysaloides*. The indument on the lower surface of the leaflets of *T. setifera* is very characteristic; it consists of short, pale, appressed hairs that radiate in all directions. Similar hairs in *T. chrysaloides* generally point in the same direction and give the leaflets a sericeous appearance. *Tachigali vasquezii*, a species known from two fruiting collections made in the Cenepa Basin in northern Peru, is also morphologically similar. It differs from *T. chrysaloides* in its indument: the lower surface of the leaflets is tomentulose, not appressed pubescent, and the midrib on the lower surface has short, erect hairs. Flowers are not yet known and stipules are not present on the two existing fruiting collections. Common names are "wantsun" (Shuar name in Ecuador) and "cano jihui" (Chacobo name in Bolivia). Some specimens had been previously annotated as *Sclerolobium chrysophyllum* Poepp., *S. rugosum*, and *T. vasquezii*, and duplicates may have been distributed under those names.

Distribution. Tachigali chrysaloides has been reported from the following areas: western Brazil (Acre, Mato Grosso, Rondônia), and adjacently in Bolivia, and along the eastern foothills of the Andes in Ecuador; a single collection is known from the Department of Loreto in Peru.

IUCN Red List category. The few known collections of this species are scattered over a large area, and a listing of Least Concern (LC) seems appropriate (IUCN, 2001).

Paratypes. BOLIVIA. **Beni:** prov. Vaca Diez, vic. of Chacobo village Alto Ivon, *Boom 1237* (NY), *1307* (NY), *1458* (MG, MO, NY). BRAZIL. **Acre:** BR-317, km 67, fl., *Rego & das C. da S. Waltir 926* (MO); Mun. Brasiléia, Reserva Extr. Chico Mendes, *Cid et al. 10140A* (INPA); Mun. Senador Guiomard, *Cid Ferreira et al. 10265* (INPA, MO). **Mato Grosso:** Aripuena, *Monteiro, Leite, Gomes & Miranda 1148* (INPA); same loc., *Silva & Pinheiro 1266* (NY), *Silva & Pinheiro 1322* (INPA, MG), *Silva & Rosario 1809* (NY). **Rondônia:** Mun. Guajira-Mirim, Chapada dos Pacaas Novos, *Cid Ferreira 8768* (INPA, MEL, MO). ECUADOR. **Morona-Santiago:** Bomboiza, alrededores de la Mision Salesiana, *Neill, Baker, Palacion & Cerón 7441* (MO); Limon Indanza, valley of Rio Coangos, *Neill & Manzanares 13212* (MO). **Zamora-Chinchipe:** Nambija, fl., *Quizhpe, Pardo, Granda & Cobos 892* (MO); in the vic. of the mining camp at Rio Tundaime, *van der Werff, Gray, Ronquillo & Quizhpe 19523* (MO). PERU. **Loreto:** Prov. Maynas, Rio Itaya, *Vasquez & Jaramillo 5740* (MO).

12. Tachigali chrysophylla (Poepp.) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 15: 1254. 1993. Basionym: *Sclerolobium chrysophyllum* Poepp., Nov. Gen. Sp. Pl. 3: 60. 1845. TYPE: Brazil. *Poeppig 2666* (holotype, W not seen; isotype, P!).

Sclerolobium sericeum Tul., Arch. Mus. Hist. Nat. 4: 125. 1844, non *Tachigali sericea* Tul. TYPE: Brazil. *Poeppig 2666* (holotype, P!).

Discussion. Tachigali chrysophylla is best recognized by the combination of its domatia (these cylindrical and moderately swollen and often beyond the first pair of leaflets) and leaflets with a strongly unequal base and sericeous lower surface. Stipules, when present, are pectinate. Petals are glabrous and linear. Most collections are from terra firme forest. The majority of collections are from Peru and Venezuela, with single collections from Brazil and Ecuador. This distribution pattern suggests that other collections from Colombia and Brazil remain unidentified or are misidentified. It is also possible that more than one species is filed under this name; the specimens are rather variable.

Poeppig (1845) was not aware of Tulasne's publication (Tulasne, 1844). Both Poeppig and Tulasne recognized *Poeppig 2666* as an undescribed species and both described it independently with the same collection as type (but with different holotypes).

Selected specimens examined. BRAZIL. **Amazonas:** Mun. Jutai, *Cid et al. 8313* (INPA, MO). ECUADOR. **Pastaza:** Moretococha, *Gudiño 1382* (MO). PERU. **Amazonas:** Rio Cenepa, *Ancuash 1242* (MO, US). **Loreto:** Pucallpa, *Lao 6* (F, NY, US). **San Martín:** Lamas, below English Evangelical Mission, *Belshaw 3477* (GH, MO, NY, US). **Ucayali:** Km 37, Carretera Pucallpa–San Alejandro, *Simpson & Schunke 1. 36* (NY). VENEZUELA. **Amazonas:** Atures, Puerto Ayacucho, *Huber & Cerda 1460* (NY, US, VEN). **Bolívar:** Reserva Forestal La Paragua, *Blanco 647* (NY).

13. Tachigali colombiana Dwyer, Brittonia 14: 56. 1962. TYPE: Colombia. Valle: Bahia de Buenaventura, *Cuatrecasas 19903* (holotype, F not seen; isotypes, MO!, US!).

Discussion. This is the only species of *Tachigali* occurring in the Pacific lowlands of Colombia west of the Andes. It is a tall tree, 25–30 m. Domatia are lacking and stipules were absent from all specimens seen. On the leaflets, secondary veins are impressed on the upper surface and prominently raised on the lower surface. The hypanthium is strongly oblique. Petals are ca. 5 × 2 mm and pubescent near the base. Stamens are dimorphic, with three stamens shorter and thicker than the others. The flowers are sessile or nearly so. *Tachigali colombiana* is known from Valle and Chocó departments at altitudes from 10–150 m.

Selected specimens examined. COLOMBIA. **Chocó:** Quibdo–Istmina Rd., 28–35 km S of Quibdo, *Gentry & Brand 36800* (INPA, MO). **Valle:** Bajo Calima, *Murphy et al. 532* (MO, US).

14. Tachigali davidsei Zarucchi & Herend., Fl. Venez. Guayana 4: 116. 1998. *Sclerolobium aureum* var. *grandiflorum* Dwyer, Lloydia 20: 81. 1957, non *Tachigali grandiflora* Huber, 1909. TYPE: Venezuela. Río Orinoco, *Chaffanjon 217* (holotype, P!).

Discussion. *Tachigali davidsei* can be readily identified by the golden-brown indument on twigs, inflorescences, and flowers; the lack of domatia; raised tertiary venation on the upper surface of the leaflets; the pectinate stipules (when present); and the symmetrical hypanthium. The base of the leaflets is symmetrical. Stamens are monomorphic; petals are ca. 5 × 2 mm.

Distribution. This species is fairly common in Venezuela along the Orinoco and its tributaries (Amazonas, Anzoátegui, Apure, Bolívar, Delta Amacuro, Guárico, and Monagas) and grows mostly along rivers or on the banks of lakes.

Selected specimens examined. VENEZUELA. **Amazonas:** Puerto Ayacucho, *Maguire et al. 36183B* (NY, US). **Anzoátegui:** Caño Mamo, *Colonello 1239* (MO). **Apure:** Río Arauca, *Davidse & Gonzalez 13387* (MO, US). **Bolívar:** 31 km W of Caicara, *Davidse 4362* (MO, NY, US). **Delta Amacuro:** Mpio. Casacoima, *Diaz et al. 6778* (MO). **Guárico:** Estación Biológica Calabozo, *Aristeguieta 5111* (COL, F, NY, VEN).

15. Tachigali dwyeri (R. S. Cowan) Zarucchi & Herend., Fl. Venez. Guayana 4: 116. 1998. Basionym: *Sclerolobium dwyeri* R. S. Cowan, Mem. New York Bot. Gard. 10: 83–85. 1961. TYPE: Venezuela. Amazonas: Río Guainia, Cano Pimichin, *Maguire et al. 41831* (holotype, US not seen; isotypes, GH!, MO!, NY!, P!).

Discussion. *Tachigali dwyeri* is best recognized by the combination of small flowers with petals (ca. 2 × 1.5 mm) about as large as sepals, sparse indument on inflorescences and flowers, terete domatia, and 4 to 5 pairs of leaflets. The base of the leaflets is somewhat asymmetrical but not strongly so. Stipules are pinnate with linear lobes but infrequently present. Stamens are monomorphic. The petals have a few hairs in the center near the base but are otherwise glabrous. *Tachigali dwyeri* is known from three collections in Amazonas, Venezuela: one from the Río Guainía, one from the Río Yatua, and one from the Río Casiquiare. The latter is sterile but has the characteristic stipules of *T. dwyeri*.

Additional specimens examined. VENEZUELA. **Amazonas:** Río Pacimoni, *Maguire et al. 41652* (GH, MO, NY, R); Solano, Bajo Casiquiare, *Williams 14734* (US).

16. Tachigali eriopetala (Ducke) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 399. 2007. Basionym: *Sclerolobium eriopetalum* Ducke, Arq. Inst. Biol. Veg. 2: 41. 1935. TYPE: Brazil. Amazonas: "prope Manaus silva non inundabili circa cataractas fluminis Taruma," *Ducke s.n. (RB 24296)* (holotype, RB not seen; isotypes, K!, P!).

Discussion. This species is best recognized by its elliptic leaflets with dense, appressed to sericeous indument, absence of domatia, and, in flowering specimens, absence of stipules; small, entire, revolute bracts are sometimes present in the inflorescences. Petals are linear and densely pubescent. The base of the leaflets varies from obtuse to rounded and is only slightly asymmetrical. Specimens collected in the Reserva Florestal de Curua-Una in Pará are also included here. These specimens have larger, oblong leaflets with more pairs of secondary veins (6 to 8 in typical *Tachigali eriopetala*, ca. 10 in specimens from Curua-Una) and large, revolute stipules at the base of the leaves. Most of these specimens are sterile. The few fertile collections from this area have leaflets much like typical *T. eriopetala* and, with the exception of *Cavalcante 1510*, do not have stipules at the base of the leaves. *Tachigali eriopetala* has been reported from terra firme forest in Amazonas and Pará. It is similar to *T. hypoleuca* (Benth.) Zarucchi & Herend., which differs in having foliose stipules and being restricted to flooded forest. It can also be confused with *T. goeldiana* (Huber) L. F. Gomes da Silva & H. C. Lima, a species with glabrous petals, floral bracts longer than the buds, and leaflets with more (10 to 15) pairs of secondary veins, and that grows in flooded forest. Several specimens placed in *T. eriopetala* previously had been annotated by others as *Sclerolobium chrysophyllum*, a species with domatia and pectinate stipules.

Selected specimens examined. BRAZIL. **Amazonas:** Mun. Maues, *Zarucchi et al. 3117* (INPA, MG, MO). **Pará:** Santarem, *Cavalcante & Silva 1510* (IAN, MG, NY).

17. Tachigali ferruginea van der Werff, sp. nov. TYPE: Ecuador. Pastaza: 40 km S of Puyo, along rd. to Macas, near Pitirishka village, 01°48′S, 77°47′W, 26 Nov. 2000, *D. Neill 12932* (holotype, MO!; isotypes, K!, NY!). Figure 9.

A congeneris foliolis magnis basi rotundatis vel cordatis, nervis lateralibus numerosis et indumento ferrugineo recedit.

Tree, to 20 m, twigs ferruginous pubescent, the hairs partly erect, partly appressed ascending.

Figure 9. *Tachigali ferruginea* (holotype).

Stipules pectinate, the lower lobes again pectinately divided, densely pubescent. Incomplete leaf with rachis ca. 40 cm, 5 pairs of leaflets, the rachis ferruginous pubescent, strongly sulcate on the lower half, and hollow, but domatia not abruptly swollen, the leaflets from 16 × 7 cm (basal ones) to 30 × 10 cm (distal ones), oblong or oblong-obovate, the base symmetrical, rounded to cordate, the tip abruptly acute, the upper surface sparsely appressed pubescent, the indument denser on the midrib and this also with scattered, erect hairs, lower surface densely greenish brown pubescent, the hairs appressed and completely covering the surface, the primary and secondary veins with some erect, ferruginous hairs; lateral veins 20 to 25 on each side. Incomplete inflorescences to 30 cm, ferruginous pubescent as on the twigs, paniculately branched, the ultimate, flower-bearing parts 4–6 cm; bracts not seen, caducous. Flowers densely grouped together, sessile, symmetrical, hypanthium and lobes densely pubescent, the lobes erect, ovate, ca. 3 × 2 mm, the cup 1.5–2 mm, symmetrical; petals linear, glabrous, 3–4 mm, stamens 10, equal, ca. 4 mm, with some stiff, ferruginous hairs near the base; ovary ca. 2 mm, densely ferruginous pubescent, style slender, ca. 2 mm, glabrous. Ovary attached in the center of the hypanthium. Fruit laterally compressed, ca. 16 × 4 cm, with 1 central seed.

Discussion. *Tachigali ferruginea* is only known from the flowering type collection, gathered in secondary forest along a road; a second, fruiting collection (*Neill & Ceron 7598*) possibly belongs here. The old flowers are reddish brown. The collector noted that the leaf petioles are inhabited by fierce, stinging ants. The new species can be readily recognized by its large leaflets with many pairs of secondary veins, its ferruginous indument, its pectinate stipules, and its symmetrically based leaflets. The fruiting collection consists of fallen fruits and leaflets and thus does not show the ferruginous indument on the twigs or leaf stalk, presence or absence of domatia, or stipules—characters required to confidently name the collection to species. It is largely placed here because of the number of secondary veins (20 to 25) and the symmetrical base of the leaflets. The indument on the old leaflets is dense, but partly worn off and paler than on the type specimen. According to the collectors, the fruiting tree appeared to be monocarpic and was dying. Several other species in Peru and adjacent Brazil also have large, densely pubescent leaflets, linear petals, and a symmetrical hypanthium, but these species differ in stipule type (entire and revolute), pilose petals, general indument type, lower number of secondary veins per leaflet, or lack hollow, ant-inhabited leaf rachises. The type collection was initially identified as *Tachigali formicarum* Harms by the collector, and duplicates may have been distributed with this identification.

IUCN Red List category. *Tachigali ferruginea* is only known from two recent collections and is listed as Data Deficient (DD) (IUCN, 2001).

Paratype. ECUADOR. **Napo:** Reserva Biologica Jatun Sacha, 01°04′S, 77°36′W, 17–23 Jan. 1987, *D. Neill & C. Ceron 7598* (MO).

18. Tachigali formicarum Harms, Verh. Bot. Vereins Prov. Brandenburg 48: 164, 1907. TYPE: Peru. Loreto: Tarapoto, *Ule 6538* (holotype, B†; isotypes, K!, L!).

Tachigali tessmannii Harms, Notizbl. Bot. Gart. Berlin-Dahlem 9: 967. 1926. Syn. nov. TYPE: Peru. Loreto: *Tessmann 4753* (holotype, B†, photo MO!).

Discussion. This species is easily recognized by the large, semiterete domatia, flowers with petals about the same size as sepals, petals with long yellow hairs, and the generally large leaflets. The yellow hairs on the petals are readily visible in opening flowers. The base of the leaflets is asymmetrical. Stamens are monomorphic. The hypanthium is symmetrical. Stipules are caducous and were absent from all specimens seen in this study. A fruiting, leafless collection (*Morawetz 13-24988*, K) seems to belong here, suggesting the species is monocarpic. Although many species of *Tachigali* have ant-inhabited domatia, the presence of aggressive ants is, in most species, only infrequently mentioned, but nearly all collections of *T. formicarum* mention the presence of stinging ants ("leaves full of tangarana ants which sting painfully," *Woytkowski 34445*; "hormigas que pican fuerte," *Schunke V. 5255*), thus the epithet of this species is well chosen. Harms distinguished *T. tessmannii* from *T. formicarum* by the more asymmetrical base of the leaflets of the latter. Collections cannot be separated based on this character alone, and therefore *T. tessmannii* is here placed in synonymy.

Selected specimens examined. PERU. **Amazonas:** Río Santiago, Comunidad Caterpiza, *Huashikat 2306* (MO). **Huánuco:** Distr. Calleria, Km 90 San Alejandro, *Schunke V. 5255* (F, US). **Loreto:** Río Marañon, just above Pongo de Manseriche, *Wurdack 2287* (F, GH, US). **Pasco:** Palcazu, Río Icozacin, *Foster & d'Achille 10213* (MO). **Ucayali:** Km 88, Pucallpa–Tongo Maria Rd., *Gentry et al. 36368* (MO).

19. Tachigali fusca van der Werff, sp. nov. TYPE: Brazil. Acre: Mun. Tarauaca, Rio Tarauaca, seringal Universo, 20 Sep. 1994, *D. Daly, M.*

Silveira, R. Saraiva, L. A. Lima & N. Goldstein 8264 (holotype, NY!; isotype, MO!). Figure 10.

Inter species domatiis et petalis linearibus praeditas floribus glabris et indumento ramulorum inflorescentiarumque fusco diversa est.

Tree, to 22 m; twigs angular, densely fuscous tomentulose to tomentose, the surface covered by the indument. Leaves (often incomplete on herbarium specimens) to 70 cm, with 4 to 12 pairs of leaflets; petiole 4–6 mm diam., the domatia ca. 4 cm from the base, swollen, cylindrical, 8–10 mm diam., 2.5–8 cm, below the first pair of leaflets; leaflets 15–18 × 4–5 cm, oblong, the base unequal, with 1 lobe cordate, the other obtuse, the tip acute, the upper surface glabrous, lower surface densely appressed pubescent and with erect fuscous hairs along the major veins; secondary veins 12 to 15 pairs per leaflet; petiolules 4–5 mm, with a similar indument to that on the leaf rachis. Stipules not seen. Inflorescences profusely branched, densely fuscous pubescent, 15–25 cm, the ultimate flower-bearing branchlets 4–7 cm; bracts not seen. Flower buds turbinate, with a few hairs at the very base, otherwise glabrous; pedicels less than 1 mm, pilose. Hypanthium ca. 1 × 1.5 mm, glabrous. Sepals glabrous on both surfaces except for the ciliate margin, ca. 2 mm, concave; petals linear, slightly widened in the distal half, glabrous; stamens equal, ca. 3 mm, moderately brown pubescent on the proximal 1/3. Pistil ca. 2 mm, densely fuscous pubescent, the style slender, glabrous, about as long as the ovary. Hypanthium symmetrical or nearly so. Fruits (immature) ca. 7 × 1.5 cm.

Discussion. *Tachigali fusca* is easily recognized by the combination of glabrous flowers, large, densely pubescent leaflets, the size of the domatia, and the fuscous indument. The few other species of *Tachigali* with glabrous flowers all have much smaller leaflets that are glabrous or sparsely pubescent; none have a fuscous indument on the twigs and inflorescences. Only *T. ferruginea* shares large, oblong, densely pubescent leaflets, linear petals, a symmetrical hypanthium, and the presence of domatia in the leaf petioles with *T. fusca*, but the former has uniformly pubescent flowers, a symmetrical base of the leaflets, and a ferruginous indument, among other differences. *Tachigali fusca* is only known from three collections. The two paratypes are in fruit and have a less dense indument on the leaflets. Both have a fuscous indument, long domatia, and leaflets with an unequal base. One of the paratypes has some hairs on the hypanthium, while the hypanthium is glabrous in the other paratype.

IUCN Red List category. Because this species is only known from three recent collections, it is listed as Data Deficient (DD) (IUCN, 2001).

Paratypes. BRAZIL. **Acre:** Mun. de Mancio Lima, margen direita ao alto rio Moa, *Cid Ferreira et al. 10032* (INPA, NY); Mun. de Cruzeira do Sul, seringal Iracema, *Cid Ferreira et al. 10781* (MEL).

20. Tachigali glauca Tul., Arch. Mus. Hist. Nat. 4: 165. 1844. TYPE: "Guiana Gallica" [French Guiana], *LePrieur 336* (holotype, P!; isotypes, L!, P!).

Sclerolobium myrmecophilum Ducke, Arch. Jard. Bot. Rio de Janeiro 1: 30. 1915. *Tachigali myrmecophila* (Ducke) Ducke, Arch. Jarb Bot. Rio de Janeiro 3: 91. 1922. Syn. nov. TYPE: Brazil. Pará: Belem, *Ducke s.n. (MG 15659)* (holotype, MG not seen; isotypes, BM!, R!, US!).

Discussion. Characteristic is the sparsely pubescent inner surface of the petals, with only some hairs in the basal, central part. Other species with a sericeous lower surface of the leaflets and domatia have a dense indument on at least the lower half of the petals (often on the lower 2/3). *Tachigali glauca* has slightly dimorphic stamens, foliose stipules (although these are infrequently present), terete domatia, and petals ca. 4 mm. The base of the leaflets is asymmetrical but not strongly so. This species is quite similar to *T. argyrophylla* and differs mainly in the sparse indument on the inner surface of the petals.

The name *Tachigali glauca* has not been used since Tulasne (1844); Dwyer (1954) did not mention it. No differences between this species and *T. myrmecophila* were found in this study, and therefore *T. myrmecophila* is placed in synonymy.

Distribution. *Tachigali glauca* has been reported from Pará, with two collections from Amapá, two from French Guiana, and one from Roraima.

Selected specimens examined. BRAZIL. **Amapá:** Serra do Navio, *Cowan 38220* (IAN, US). **Pará:** Jacunda, Rio Tocantins, *Froes 27090* (IAN, US). **Roraima:** Estrada Manaus Caracarai, Km 343, *Steward et al. 106* (INPA, US). FRENCH GUIANA. *Leprieur 336* (L, P). GUYANA. ca. 20 km S of Mabura Hill, *Polak 321* (MO).

21. Tachigali goeldiana (Huber) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 399. 2007. Basionym: *Sclerolobium goeldianum* Huber, Bol. Mus. Paraense Hist. Nat. 6: 78. 1910. TYPE: Brazil. Pará: Rio Capim, *Huber s.n. (MG 692)* (holotype, MG!; isotype, BM!).

Discussion. *Tachigali goeldiana* is a small tree of riverbanks and flooded forests that is best recognized by its stiff leaflets with a symmetrical base and a dense, appressed, golden or silvery lower surface of the leaflets. Domatia are absent. Stipules are pectinate but rarely present. Petals are linear and glabrous. The inner surface of the sepals is glabrous. For their size, the leaflets have many lateral veins (10 to 15 on each side). *Tachigali goeldiana* has been confused with *T.*

Figure 10. *Tachigali fusca* (holotype).

hypoleuca, also a species of flooded forests with sericeous lower leaflet surface. However, *T. hypoleuca* differs in its foliaceous stipules, pilose petals, and fewer (up to six) pairs of lateral veins. *Tachigali goeldiana* is known from Pará, Amazonas, and Roraima in Brazil.

Selected specimens examined. BRAZIL. **Amazonas:** Ilha da Costa, Arirarra, *Poole 1719* (INPA, MG, MO, NY, US). **Pará:** Rio Capim, lg. Caju, *Froes 24113* (IAN, NY, RB, US). **Roraima:** Rio Branco, at mouth of Rio Itapera, *Mori et al. 20414* (INPA, MO, NY).

22. Tachigali grandistipulata Harms, Notizbl. Königl. Bot. Gart. Berlin 6: 304. 1915. TYPE: Brazil. Roraima: Rio Branco, Surumu, Serra de Mairary, *Ule 8399* (holotype, B†; isotypes, F! [fragm.], K!, L!).

Discussion. This species resembles *Tachigala rigida* Ducke, but differs in its smaller flowers (petals ca. 5 mm vs. petals 8–9 mm in *T. rigida*) and monomorphic stamens (4–6 mm). The base of the leaflets is obtuse to rounded and more asymmetrical than in *T. rigida*. Domatia are present and semiterete. Stipules are foliose and large, to 5 × 3 cm in the specimen at K. The lower leaf surface is completely covered by the appressed indument. The stamens are about as long as the petals, while in *T. rigida* stamens are clearly longer than the petals. *Tachigali grandistipulata* is only known from the type collection from Roraima.

23. Tachigali guianensis (Benth.) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Basionym: *Sclerolobium guianense* Benth., Hooker's J. Bot. Kew Gard. Misc. 2: 237. 1850. TYPE: Guyana. *Rob. Schomburgk, 2nd coll. 598* (lectotype, designated by Zarucchi & Herendeen in Brako & Zarucchi, 1993: 1254. K not seen; duplicates, BM!, P!).

Sclerolobium radlkoferi Rusby, Mem. Torrey Bot. Club 6: 26. 1896. *Sclerolobium guianense* var. *radlkoferi* (Rusby) Dwyer, Lloydia 20: 98. 1957. Syn. nov. TYPE: Bolivia. Betw. Tipuani & Guanai, *Bang 1690* (holotype, NY not seen; isotypes, GH!, K!).

Sclerolobium uleanum Harms, Verh. Bot. Vereins Brandenburg 48: 168. 1907. *Sclerolobium tinctorium* var. *uleanum* (Harms) Dwyer, Lloydia 20: 95. 1957. *Tachigali uleana* (Harms) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Syn. nov. TYPE: Peru. Tarapoto, *Ule 6450* (holotype, B†; isotype, K!).

Sclerolobium subbullatum Ducke, Arq. Inst. Biol. Veg. 2: 42. 1935. *Tachigali subbullata* (Ducke) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 400. 2007. Syn. nov. TYPE: Brazil. Amazonas: "ca. Esperança ad ostium fluminis Javary," *Ducke s.n. (RB 21298)* (holotype, RB not seen; isotypes, K!, P!, US!).

Discussion. Tachigali guianensis is readily recognized by the pilose indument on twigs, leaves, and inflorescences; this is especially noticeable on young growth. The base of the leaflets is asymmetrical, stipules are pectinate, and the great majority of the specimens turn black upon drying. Petals are linear and glabrous, and domatia are lacking. Collections from Peru and Bolivia have a denser indument and have been described as *Sclerolobium radlkoferi* (from Bolivia) and *S. uleanum* Harms (from Peru). In this study, these specimens with a denser indument are included in *T. guianensis*. Ducke said *S. subbullatum* differed from *T. guianensis* in its larger and more or less bullate-rugose leaflets. However, specimens with larger and wider leaflets occur throughout the range of *T. guianensis*, and while some collections from Ecuador and Peru (*Neill 14618, Grandez 1395*) have bullate leaflets, others have flat leaflets. The concept of *T. guianensis* here presented is thus fairly broad, and more detailed studies may lead to the reestablishment of some synonyms as distinct species.

Distribution. Tachigali guianensis has a wide distribution and has been collected in Brazil (Amapá, Amazonas, Pará, and Roraima), Guyana, French Guiana, Suriname, Venezuela (Amazonas, Bolívar, and Delta Amacuro), Colombia (Putumayo), Peru (Cusco, Loreto, Madre de Dios), and Bolivia.

Selected specimens examined. BOLIVIA. **Beni:** Vaca Diez, *Boom 4368* (MG, MO, NY). **La Paz:** Copacabana (10 km S of Mapiri), *Krukoff 11020* (NY). **Pando:** 35 km N of Puerto America, *Jardim 2442* (MO, NY). **Santa Cruz:** PN Noel Kempff, *Quevedo 2697* (MO, NY). BRAZIL. **Amapá:** Camaipi, on rd. to Rio Preto, *Mori et al. 16545* (MG, NY). **Amazonas:** Mun. Humayta, near Livramento, *Krukoff 6903* (MO, NY, US). **Pará:** Rio Jipuru, Ilha do Marajo, *Rabelo & Rosa 3605* (MO, NY). **Roraima:** Ilha de Maraca, *Ratter et al. 6318*1 (NY). COLOMBIA. **Putumayo:** entre Umbria y Puerto Asis, *Cuatrecasas 10543* (COL, F, NY, US). ECUADOR. **Morona-Santiago:** Centro Shuar Yunkumas, *Neill & WSF Dendrology Course 14618* (MO). **Pastaza:** Puerto Sarayacu, *Lugo 3918* (MO). FRENCH GUIANA. Criques Ouaqui, *Lemoine 7820* (NY). GUYANA. Mt. Roraima, *Tate 243* (NY). PERU. **Cusco:** Distr. Camanti, Maniti, *Timana 1159* (MO). **Loreto:** Orilla San Miguel, Río Yarapa, *Grandez 1395* (INPA). **Madre de Dios:** Tambopata, Explorer's Inn, *Gentry et al. 51539* (MO). SURINAME. Blakawatra creek, 10 km E of Jodensavannne, *Schulz 8238* (NY). VENEZUELA. **Amazonas:** Puerto Ayacucho, Río Cataniapo, *Castillo 2191* (MO, NY). **Bolívar:** 7 km NE of Ciudad Piar, *Liesner & Gonzalez 11485* (MO, US). **Delta Amacuro:** Depto. Tucupita, 5–14 km ESE of Los Castillos de Guayana, *Davidse & Gonzalez 16266* (MO, NY, US).

24. Tachigali hypoleuca (Benth.) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Basionym: *Sclerolobium hypoleucum* Benth., Hooker's J. Bot. Kew Gard. Misc. 2: 236. 1850. TYPE: Brazil. Amazonas: woody hills at Barra do Rio Negro, *Riedel s.n.* (holotype, K!).

Discussion. This species is best recognized by the sericeous indument of the leaflets, its plane, foliaceous stipules, the nearly symmetrical base of the leaflets, and the linear, pilose petals. Domatia are absent. Nearly all collections are from flooded forest. It is similar to *Tachigali eriopetala*, which is a tree from terra firme forest with caducous stipules and which has small, revolute bracts in the inflorescences. Differences with *T. goeldiana* are discussed under that species. *Tachigali hypoleuca* is a rather common species from Amazonas and Roraima in Brazil. A collection from Venezuela (*Stergios 9703*, from Apure) is tentatively placed here; in leaf characters and stipules it agrees with *T. hypoleuca*, but it has small swollen domatia at the base of the leaf rachis; flowers are too old to assist in identification.

Selected specimens examined. BRAZIL. **Amazonas:** Manaus, Igarape da Cachoeira Grande, *Ducke 523* (F, GH, NY, R). **Roraima:** Rio Xeriuini, *Pires et al. 13961* (IAN, US).

25. Tachigali inconspicua van der Werff, sp. nov. TYPE: Ecuador. Napo: Estación Biológica Jatun Sacha, 450 m, 24–31 Dec. 1986 (fl.), *D. Neill & M. Asanza 7565* (holotype, MO!; isotype, K!). Figure 11.

Tachigali amplifoliae similis, sed ab ea foliolis oblongis vel elliptico-oblongis basi plus minusve symmetricis et nervis lateralibus 8 ad 11 (nec 5 ad 7) recedit.

Large trees, 30–40 m; twigs angular, glabrous. Stipules persistent, 1- to 3-foliose, 1–2 × 1–2 cm, the 2 basal lobes when present smaller. Leaf rachis 20–30 cm, sparsely appressed pubescent, somewhat canaliculate, especially just below the attachment of the leaflets; domatia lacking; leaflets 5 to 8 pairs, petiolules 5–8 mm, sparsely appressed pubescent, oblong to elliptic-oblong, 8–15 × 2.5–5 cm, chartaceous, the base ± symmetrical, apices acute or acuminate, acumen ca. 1 cm, secondary veins 8 to 11 pairs per leaflet. Inflorescences paniculate, moderately to densely appressed pubescent, 15–20 cm, the ultimate, flower-bearing racemes to 10 cm; bracts caducous, not seen; pedicels 0.6–1 mm, buds uniformly grey appressed pubescent. Hypanthium turbinate, symmetrical, ca. 1 × 1.5 mm, sepals ca. 2 mm, elliptic, concave, the inner surface appressed pubescent; petals ca. 2 mm, linear, densely yellow pubescent; stamens equal, 4–5 mm, the filaments yellow pubescent on the basal quarter; ovary 1.5–2 mm, covered with stiff, brown hairs. Legume samaroid, ellipsoid, ca. 8 × 2.5 cm, 1-seeded.

Discussion. As the name suggests, *Tachigali inconspicua* does not have striking features. It belongs in the group of species with linear, pilose petals, and a symmetrical hypanthium that lacks domatia. In this group it stands apart by the combination of rather large, foliose stipules, yellow hairs on the petals, and a more or less symmetrical base to the leaflets. It is closest to *T. amplifolia*, which differs in its larger, broader leaflets (to 20 × 11 cm) with fewer secondary veins (5 to 7 vs. 8 to 11) and a more asymmetrical base. *Tachigali inconspicua* is known from the Andean foothills from central Ecuador (Jatun Sacha) to the Río Cenepa Basin in northern Peru. All collections known to date are from tall trees, most estimated as 30 m and one as 40 m tall. Eighteen duplicates were made of the type collection, all but the holotype were distributed as *T. paraensis*.

IUCN Red List category. Given its distribution in Amazonian Ecuador and Peru, this species is listed as Least Concern (LC) (IUCN, 2001).

Paratypes. ECUADOR. **Napo:** Orellana Canton, 5 km N de Coca, *W. Palacios 3179* (MO, NY); Huaorani Ethnic Reserve, vic. of the Dicaro community, *N. Pitman et al. 4305* (MO). **Morona-Santiago:** carretera Bomboiza–Gualaquiza, a 8 km de la Mision, fl., *J. Zaruma 359* (MO). **Zamora-Chinchipe:** Río Nangaritza, downriver from Miazi to Pachicutza, fl., *D. Neill & W. Palacios 9708* (MO); same locality, fr., *D. Neill & W. Palacios 9707* (MO). PERU. **Amazonas:** S of Huampami trail, S of Rio Cenepa, fl., *B. Berlin 1660* (MO, US); Condorcanqui prov., El Cenepa, Comunidad de Mamayaque, fr., *R. Roja, A. Peña & E. Chávez 345* (MO). **Loreto:** Fundo Acosta, Río Maniti, *F. Encarnacion E-1010* (MO, NY, US); Iquitos, Alto do Itaja, *A. Ducke 1814* (F, IAN, MG, US).

26. Tachigali leiocalyx (Ducke) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 399. 2007. Basionym: *Sclerolobium leiocalyx* Ducke, Bol. Tecn. Inst. Agron. N. 2: 19. 1944. TYPE: Brazil. Amazonas: São Paulo de Olivença, *Ducke 1028* (lectotype, designated here, RB not seen; duplicates, GH!, K!, MO!, R!).

Discussion. This species is easily recognized by the glabrous sepals, linear, pubescent petals, and the coriaceous, elliptic to elliptic-ovate leaflets. The base of the leaflets is obtuse to rounded, weakly asymmetrical. Domatia are lacking, and stipules have not been seen on the few known specimens. According to the labels, the three known collections were all made in caatinga forest near São Paulo de Olivença. Silva and Lima (2007) indicated that the holotype is in RB; unfortunately, a lectotype has to be selected because Ducke (1944) cited two collections as syntypes. Two species were distributed under *Ducke 1028*. One consists of the lectotype and isolectotypes of *Tachigali leiocalyx* (in GH, K, MO, R, and RB), while the other (in MO and US) represents *T. inconspicua*.

Figure 11. *Tachigali inconspicua* (holotype).

Additional specimens examined. BRAZIL. **Amazonas:** São Paulo de Olivença, *Ducke 1498* (NY, R); *Lima et al. 2771* (MO).

27. Tachigali longiflora Ducke, Arq. Inst. Biol. Veg. 2: 38. 1935. TYPE: Brazil. Amazonas: "São Paulo de Olivença, silva non inundabili," *Ducke s.n. (RB 24291)* (holotype, RB not seen; isotypes, K!, P!, US!).

Discussion. *Tachigali longiflora* is a striking species because of its very dark brown inflorescences and flower buds. The flowers are rather large for the genus (to 1.8 cm), and the hypanthium is unusually long and curved; this is best visible just after anthesis and after the petals have fallen. Stamens are dimorphic. Petals are large, ca. 8 × 4 mm. Stipules have not been seen for this study; Ducke mentioned he had seen one incomplete stipule, this pinnate, with narrow segments. Domatia have also not been seen in this study, though Ducke mentioned the presence of small ants in the leaf rachis. The leaflets have an asymmetrical base and vary from ovate-elliptic (basal pairs) to oblong (distal pairs).

Additional specimens examined. PERU. **Loreto:** Jenaro Herrera, *Huamantupa et al. 5358* (MO), *Daly et al. 5741* (MEL, MO), *Spichiger 1490* (MO).

28. Tachigali loretensis van der Werff, sp. nov. TYPE: Peru. Loreto: Prov. Maynas, Alpahuayo, 12 Nov. 1984 (fl.), *R. Vasquez, J. Ruiz & N. Jaramillo 5888* (holotype, MO!; isotypes, F!, K!, NY!). Figure 12.

Tachigali ptychophyscae Benth. similis, sed ab ea floribus minoribus et staminibus uniformibus recedit.

Tall trees, to 40 m; twigs terete, minutely and densely appressed pubescent. Stipules not seen; leaf rachis 10–20 cm, minutely and moderately densely appressed pubescent, terete; domatia present, terete, 1.5–3 cm, 4–6 mm diam., less than 1 cm from the base of the leaves; leaflets 4 to 6 pairs, 5–13 × 3–6 cm, ovate to elliptic, chartaceous, the upper surface glabrous, lower surface inconspicuously and moderately to sparsely appressed pubescent, the base asymmetric, the apex acute to acuminate, acumen to 1 cm, secondary veins 3 to 8 pairs per leaflet. Inflorescences paniculate, 15–25 cm, densely and minutely appressed pubescent, the ultimate racemes to 10 cm, bracteoles ca. 5 mm, narrowly ovate to linear, falling off as buds mature and absent from the base of the mature flowers; pedicels to 1 mm, buds uniformly and densely grey pubescent. Hypanthium asymmetrical; sepals broadly ovate, 2–3 × ca. 1.5 mm, pubescent on the inner surface; petals slightly longer than the sepals, to 2 mm wide, ovate, pubescent on the inner surface, glabrous on the outer surface, stamens 10, monomorphic, ca. 5 mm, pubescent near the base; ovary ca. 1.5 mm, densely pubescent, style ca. 2.5 mm, pubescent near the base, becoming glabrous distally. Fruits unknown.

Discussion. Vegetatively, *Tachigali loretensis* closely resembles *T. ptychophysca*. Both species have leaflets with few lateral veins, terete domatia close to the base of the petiole, and a similar leaflet shape. The specimens from Colombia of *T. loretensis* have more lateral veins, up to eight pairs per leaflet. However, *T. ptychophysca* differs in its larger flowers with sepals 4–5 mm, petals 5–6 mm, and stamens 10–12 mm, and in its dimorphic stamens. The appressed indument on the lower surface of the leaflets is also denser in *T. ptychophysca* and appears almost sericeous. *Tachigali loretensis* is known from three flowering collections made in non-flooded forest on white sand near Iquitos and two flowering collections from Vaupes, Colombia. Seedlings and juvenile trees have distinctly acuminate leaflets, with an acumen up to 4 cm.

IUCN Red List category. *Tachigali loretensis* does not have a wide distribution (northeast Peru and adjacent Colombia). However, sterile collections from forest plots suggest that it is locally common and it is therefore listed as Vulnerable (VU) (IUCN, 2001).

Paratypes. COLOMBIA. **Vaupes:** Río Kananari, Cerro Isibukuri, *Schultes & Cabrera 14691* (COL, GH); Río Kananari y Cerro Isibukuri, Nov., *H. Garcia-Barriga 13771* (COL, US). PERU. **Loreto:** Prov. Maynas, Mishana, Río Nanay, fl., *Gentry et al. 39315* (MO); Prov. Maynas, Alpahuayo, *Vasquez & Criollo 5761* (MO).

29. Tachigali macbridei Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Replaced synonym: *Sclerolobium rigidum* J. F. Macbr., Field Mus. Nat. Hist., Bot. Ser. 13: 201. 1943, non *Tachigali rigida* Ducke, 1938. TYPE: Peru. Loreto: Pumayacu, *Klug 3239* (holotype, F not seen; isotypes, GH!, MO!).

Discussion. This species can be easily confused with *Tachigali chrysophyllum*; it is best separated from this species by the erect indument on the leaf rachis. In this character, it approaches *T. guianense*, but the latter lacks the sericeous indument on the lower surface of the leaflets. Stipules are pectinate. Domatia are often present in large leaves, but lacking in small leaves. The base of the leaflets is asymmetrical. Petals are linear and glabrous. Most collections are from upland forest. *Tachigali macbridei* is known from six departments in Peru, with a few collections from adjacent Brazil (Acre),

Figure 12. *Tachigali loretensis* (holotype).

Several collections from Peru (Amazonas) have nearly sessile leaflets with a cordate base clasping the rachis.

Selected specimens examined. BRAZIL. **Acre:** near mouth of Rio Macauhan, *Krukoff 5469* (GH, MO, NY). PERU. **Amazonas:** Distr. Imaza, Comunidad Agauruna de Kusu-Listra, *Diaz et al. 8220* (MO). **Cusco:** Distr. Echarate, Kiteni, *Huamantupa et al. 7225* (MO). **Huanuco:** Tingo Maria, *Asplund 13171* (NY). **Loreto:** Río Nanay, carretera de Picuruyacu, *Rimachi 4034* (MO, NY). **Madre de Dios:** Parque Nacional Manu, *Foster et al. 11850* (MO, NY). **San Martín:** Caseria Nueva Union abajo de Puerto Huicte, *Schunke I. 7936* (MO, US).

30. Tachigali macropetala (Ducke) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 399. 2007. Basionym: *Sclerolobium macropetalum* Ducke, Arq. Inst. Biol. Veg. 2: 41. 1935. TYPE: Brazil. Amazonas: "Rio Negro superioris inter Camanaos et ostium fluminis Curicuriary," *Ducke s.n. (RB 23328)* (holotype, RB not seen; isotypes, K!, P!).

Discussion. Tachigali macropetala is a very distinct species and is easily recognized by the essentially glabrous sepals (but with a fringe of hairs along the margin), the symmetrical hypanthium, rather long pedicels (3–6 mm), elliptic petals clearly longer than the sepals, and leaflets with a symmetrical base and immersed venation on the upper surface. Domatia are lacking. Stipules are 2-foliose, 1–2 cm, but rarely present. Stamens are monomorphic. All but one of the collections are from riverbanks or flooded forest along the upper Rio Negro.

Selected specimen examined. BRAZIL. **Amazonas:** Rio Curicuriary, *Ducke 42* (F, NY).

31. Tachigali macrostachya Huber, Bol. Mus. Paraense Hist. Nat. 5: 387. 1909. TYPE: Brazil. Pará: "in ripis insulae Veneza fl. Mapuera," *Ducke s.n. (MG 9030)* (holotype, MG not seen; isotype, BM!).

Discussion. This is a very distinct species because of its large, oblong-ovate, bullate leaflets with a cordate, symmetrical base, and large flowers with 15 or more stamens. The petals are 7–8 mm long and pubescent on the inner surface. Stamens are dimorphic. The leaf petioles are not noticeably swollen, but are hollow and inhabited by ants. Leaf rachises are strongly angular. Stipules are to 3 cm long and foliose with 1 to 2 pairs of lobes, but caducous. The hypanthium is strongly asymmetrical. *Tachigali macrostachya* is a small tree growing on riverbanks. Dwyer (1954) listed the Río Mapuera in Venezuela as the type locality, possibly misled by the actual type locality given by Huber ("in ripis insulae Veneza"). The seven collections seen in this study are all from the Rio Trombetas–Rio Mapuera area in Pará, Brazil. Ducke (1949) also reported it from the Rio Jamunda (border of Pará and Amazonas).

Selected specimen examined. BRAZIL. **Pará:** Mun. Oriximina, Rio Trombetas, *Cid et al. 1256* (INPA, MG, MO, US).

32. Tachigali melanocarpa (Ducke) van der Werff, comb. nov. Basionym: *Sclerolobium melanocarpum* Ducke, Arq. Inst. Biol. Veg. 2: 43. 1935. TYPE: Brazil. Amazonas: "prope Borba, Rio Madeira infer.," *Ducke s.n. (RB 23330)* (lectotype, designated here, RB not seen; duplicates, K!, P!).

Discussion. Tachigali melanocarpa is very similar to *T. paraensis* in leaflet shape (both have a cuneate, more or less symmetrical base), and the two are easily confused. The clearest difference seems to be found in the densely hairy, linear petals: with yellow hairs in *T. melanocarpa* and white hairs in *T. paraensis*. On average, the leaflets are larger in *T. paraensis*. Also, in *T. melanocarpa* stipules fall early from young branchlets, whereas in *T. paraensis* stipules are frequently persistent, unifoliate, and foliose, to 2.5 cm. In both species, small, revolute bracts are occasionally present along the inflorescences. Domatia are absent. The hypanthium is symmetrical. Ducke (1935) also stated that the rachis and leaf petiole are canaliculate in *T. melanocarpa*, although this was not apparent on material seen in this study. The leaf rachis is somewhat flattened in *T. melanocarpa* and terete in *T. paraensis*, but this single character difference hardly warrants recognition of two separate species. *Tachigali melanocarpa* has been reported from Amazonas and Pará, Brazil, and occurs in terra firme forests. The protologue cites *Ducke RB 23339* as one of the syntypes, but the K and P sheets carry the number *Ducke RB 23330*. Ducke cited two collections in his description of *Sclerolobium melanocarpum*, one with flowers and one with fruits. The RB specimen of the flowering collection is designated here as the lectotype.

Selected specimens examined. BRAZIL. **Amazonas:** Reserva 1501, Projecto Fragmentos Florestais, 90 km NNE de Manaus, *Oliveira et al. 361* (INPA, MO, NY). **Pará:** Jari, Estrada entre Monte Dourado e Mungaba, *Silva 1978* (NY, US).

33. Tachigali melinonii (Harms) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Basionym: *Sclerolobium melinonii* Harms, Bot. Jahrb. Syst. 33 (Beibl. 72): 24. 1903. TYPE: French Guiana. Maroni [Saint-Laurent-du-Maroni]: *Melinon s.n. in 1861* (holotype, B†; isotype, P!).

Discussion. *Tachigali melinonii* is an inconspicuous species, but is easily recognized by its sparse, stellate indument. No other species of *Tachigali* has stellate hairs. Stipules of this species have not been seen. Domatia are lacking. Petals are slightly longer than the sepals, glabrous, linear or slightly dilated, but fall off readily and are thus often lacking on herbarium specimens. The base of the leaflets is asymmetrical. Habitat data, when given by collectors, indicate this is a species of terra firme forest. It has been reported from Suriname, French Guiana, Brazil (Amapá, Amazonas), and Peru (Loreto), but is uncommon in herbaria.

Selected specimens examined. BRAZIL. **Amapá:** Mun. Mazagão, *Silva 5525* (INPA). **Amazonas:** Reserva 1501, Projeto Fragmentos Florestais, *Mori et al. 20617* (INPA, MO). FRENCH GUIANA. Crique Passoura, Région de Kourou, *Sabatier & Prévost 3753* (NY). PERU. **Loreto:** Jenaro Herrera, *Gentry et al. 56376* (MO).

34. Tachigali micrantha (L. O. Williams) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Basionym: *Sclerolobium micranthum* L. O. Williams, Fieldiana, Bot. 31: 32. 1965. TYPE: Peru. Huánuco: *Gutierrez 44* (holotype, F not seen; isotypes, K!, US!, WIS not seen).

Discussion. This species is easily recognized by the combination of glabrous sepals, small, obovate petals shorter than the sepals, and the rather dense, erect indument on the lower surface of the leaflets. Domatia are lacking; stipules are laciniate, to 2 cm, and the base of the leaflets is obtuse to subcordate and somewhat asymmetrical. The flowers are small and clearly pedicellate, with the pedicels about as long as the flowers. For this study, only the K and US isotypes and the fruiting paratype (*Gutierrez 96*) have been seen.

35. Tachigali micropetala (Ducke) Zarucchi & Pipoly, Sida 16: 787. 1995. Basionym: *Sclerolobium micropetalum* Ducke, Bol. Tecn. Inst. Agron. N. 2: 20. 1944. TYPE: Brazil. Amazonas: Manaus, "silva terris altis argillosis ultra Flores," *Ducke 1219* (holotype, IAN not seen; isotypes, MO!, US!).

Discussion. This species is easily recognized by the combination of small flowers and the distally glabrous sepals. Petals are elliptic, to 1 mm. Stamens are monomorphic. Stipules have not been seen on any of the flowering specimens studied, but are present on two sterile specimens (*Ducke s.n. [RB 20343], Evans & Peckham 2941*); they are densely pubescent and pectinate. Domatia are lacking. Leaflets near the base of the leaf have an asymmetrical base, whereas the distal leaflets have a more symmetrical base. The margins of the leaflets are frequently inrolled. *Tachigali micropetala* is known from the area near Manaus, from Pará in Brazil, Guyana, French Guiana, Suriname, and Venezuela.

Selected specimens examined. BRAZIL. **Amazonas:** Estrada Manaus–Itacoatiara, Km 134, *Rodrigues & Loureiro 9510* (INPA). **Pará:** Jari, Estrada entre Tinguelim e S. Miguel, *Silva 3322* (IAN). FRENCH GUIANA. **Saül:** circuit Limonade, *Moretti 713* (US). GUYANA. Moraballi Creek, *Fanshawe 571* (NY). SURINAME. Mapanagebied, *Schulz 7703* (GH, NY). VENEZUELA. **Delta Amacuro:** esteros de El Palmar, *Marcano Berti 635* (MO).

36. Tachigali odoratissima (Benth.) Zarucchi & Herend., Fl. Venez. Guayana 4: 116. 1998. Basionym: *Sclerolobium odoratissimum* Benth. in Mart., Fl. Bras. 15(2): 48. 1870. TYPE: Brazil. Amazonas: *Spruce 3057* (holotype, K!; isotype, P!).

Discussion. *Tachigali odoratissima* is best recognized by the narrow leaflets with a symmetrical base and glabrous or sparsely appressed pubescence on the lower surface. Stipules are 1- to 3-foliose and ca. 1 cm. Petals are linear and densely pubescent. Stamens are equal. Domatia are terete and generally 2–4 cm. Nearly all collections seen in this study are from flooded forests or were made along rivers. It is a common species in Brazil (Amazonas, along the Rio Negro and tributaries; one collection is known from the Rio Japura) and Venezuela (Amazonas, along the Río Negro and the Río Orinoco and their tributaries). There are a few records from Colombia (Guainía and Vichada).

Selected specimens examined. BRAZIL. **Amazonas:** Barcelos, *Duarte 7252* (F, HB, INPA, US). COLOMBIA. **Guainía:** Caño Mina, en el Río Inirida, *Espina 338* (COL). **Vichada:** Puerto Nuevo, *Daniel 127* (COL). VENEZUELA. **Amazonas:** lower part of the Río Baria, *Davidse 27598* (MO, NY).

37. Tachigali paniculata Aubl., Hist. Pl. Guiane 1: 372. 1775. TYPE: French Guiana. *Aublet s.n.* (holotype, BM!).

Tachigali trigona Aubl., Hist. Pl. Guiane 1: 374. 1775. TYPE: French Guiana. *Aublet s.n.* (holotype, BM!).

Tachigali sericea Tul., Arch. Mus. Hist. Nat. 4: 163. 1844. TYPE: Brazil. Pará: *Poeppig 3039* (lectotype, designated here, P!; duplicate, L!).

Tachigali eriocalyx Tul., Arch. Mus. Hist. Nat. 4: 164. 1844. TYPE: Brazil. Amazonas: Ega, *Poeppig 2737* (holotype, P!; isotype, L!).

Tachigali angustifolia Miq., Stirp. Surinam. Select. 13. 1851. *Tachigali paniculata* var. *angustifolia* (Miq.) Dwyer, Ann. Missouri Bot. Gard. 41: 239. 1954. TYPE: Suriname, *Kappler 1931* (holotype, U not seen).

Tachigali grandiflora Huber, Bol. Mus. Paraense Hist. Nat. 5: 388. 1909. Syn. nov. TYPE: Brazil. Pará: "in silvis ripariis ad fl. Mapuera," *Ducke s.n. (MG 8965)* (holotype, MG not seen; isotype, BM!).

Tachigali ulei Harms, Notizbl. Königl. Bot. Gart. Berlin 6: 306. 1915. Syn. nov. TYPE: Brazil. Amazonas: Rio Negro. São Joaquim, *Ule 6042* (holotype, B†; isotypes, K!, L!, MG!).

Tachigali rusbyi Harms, Bot. Jahrb. Syst. 33, Beibl. 72: 20. 1903. Syn. nov. TYPE: Venezuela. Orinoco: *Rusby 127* (holotype, B†; isotypes, GH!, MO!).

Tachigali pulchra Dwyer, Ann. Missouri Bot. Gard. 41: 249. 1954. Syn. nov. TYPE: Venezuela. Bolívar: Alto Río Paragua, *Cardona 1180* (holotype, US!).

Tachigali paniculata var. *comosa* Dwyer, Ann. Missouri Bot. Gard. 41: 240. 1954. Syn. nov. TYPE: Brazil. Amazonas: São Paulo de Olivença, *Krukoff 8851* (holotype, MO!; isotype, GH!).

Discussion. Here, *Tachigali paniculata* is accepted as a wide-ranging and variable species, known from the Guianas to Peru. It is also the most frequently collected species of *Tachigali*. Domatia are mostly absent, but sometimes present and semiterete. Of 16 collections checked from the Iquitos area, all from flooded forest, two had well-developed domatia, two had some duplicates with domatia and others without, two had the petiole bases not thickened (but ant holes were present in the petiole), and the rest had no traces of domatia or ant habitation. Therefore, presence or absence of domatia is not considered to be a diagnostic character of *T. paniculata*. Stamens are dimorphic, with three stamens shorter and falcately curved, and this character separates *T. paniculata* from *T. alba*, which has monomorphic stamens. *Tachigali alba* and *T. richardiana*, another similar species, also differ in their terete leaf rachises; these are sharply angular in *T. paniculata*. Stipules, when present, are foliose, with one or two pairs of lobes. The base of the leaflets is asymmetrical. Leaflets are often glabrous or nearly so, but sometimes may be moderately to densely appressed pubescent on the lower surface. Nearly all collections come from flooded forest, and most trees are less than 15 m tall (occasionally reaching 20 m).

However, there is so much morphological variation in *Tachigali paniculata* (as recognized in this study) that additional analysis may reveal further data sufficient to segregate more taxa from among the current synonymy.

Of the two syntypes of *Tachigali sericea*, *Poeppig 3039* has flowers and is selected as lectotype, while the specimen from the Richard herbarium (French Guiana. Herb. *Richard s.n.* [P]) has fruits. The latter has a denser indument on the leaflets.

Tachigali grandiflora differs from typical *T. paniculata* in several qualitative and quantitative characters. The leaflets are thinner, have an acuminate tip, and have the secondary and tertiary venation raised on the lower surface. The flowers are rather large, larger than average for *T. paniculata*. However, other collections placed in *T. paniculata* can have some of these characters, although not all on the same specimen. To date, not a single specimen has been seen that matches the type of *T. grandiflora*; therefore, it has been included (with some hesitation) as a synonym of *T. paniculata*.

Ule 6042 was first identified by Harms (1907) as *Tachigali paniculata* and in 1917 was promoted, without comment, to the type of *T. ulei*. In this study, Harms's first identification is accepted, and thus *T. ulei* is considered a synonym of *T. paniculata*.

Tachigali rusbyi Harms was said to differ from *T. paniculata* in its smaller flowers. The flowers certainly do not differ in size by enough to warrant recognition of a separate species. Stamens of *T. rusbyi* are less clearly dimorphic than in most collections of *T. paniculata*, but they are not equal either. Dwyer (1954) emphasized the sharply angled petioles of *T. rusbyi*, but specimens with such petioles occur throughout the range of *T. paniculata*.

Tachigali pulchra was recognized solely based on its long-stipitate ovary. Dwyer's description ("stipes ovarii 3–8 mm longus. . . ovario 1–2 mm longo. . . fructus non visus") suggests that the ovaries are long-stipitate in the flowering stage. The holotype does not show such long-stipitate ovaries in the flowers, but does have some young fruits (to 3 cm) and these are indeed rather long-stipitate. According to Dwyer, in other characters *T. pulchra* was indistinguishable from *T. rusbyi* (the types of both species were collected in the Orinoco drainage in Venezuela), and it seems best to place *T. pulchra* as a synonym of *T. paniculata*.

Most collections of *Tachigali paniculata* are from medium-sized trees in flooded forest and range from the Guianas, throughout Amazonian Brazil, to Venezuela and Peru.

Selected specimens examined. BOLIVIA. **Pando:** Río Negro near jct. with Río Abuna, *Gentry & Perry 77998* (MO). BRAZIL. **Acre:** Mun. Mancio Lima, Parque Nacional da Serra do Divisor, *Daly et al. 9012* (MO). **Amapá:** Rio Araguari, *Pires et al. 50387* (F, GH, IAN, MG). **Amazonas:** Tarumã Grande, *Keel & Guedes 281* (US). **Pará:** Mun. Oriximina, Rio Trombetas, *Cid et al. 1773* (MG, MO, US). **Rondônia:** Mun. Jaru, Km 128 estrada Cuiaba–Porto Velho, *Cid et al. 5000* (MEL). COLOMBIA. **Amazonas:** Loretoyacu River, *Schultes 8266* (COL). **Vaupes:** Mitu, lower Río Kubitu, *Zarucchi 2151* (MO, US). FRENCH GUIANA. Rivière Grand Inini, *de Granville B-3634* (HB). GUYANA. Cuyuni River, Akarabice Creek, *Tutin 440* (US). PERU. **Loreto:** Maynas, Caserio Casococha, *Revilla 1527* (MO, US). SURINAME. Lower slopes of Juliana Top, *Irwin et al. 54827* (F, US). VENEZUELA. **Amazonas:** Alto Río Orinoco betw. Tama-Tama and Esmeralda, *Maguire et al. 41550* (F, NY). **Bolívar:** Alto Río Paragua, *Cardona 1182* (US). **Delta Amacuro:** Río Amacuro, upstream from San Victor, *Steyermark 87233* (F, US).

38. Tachigali paraensis (Huber) Barneby, Brittonia 48: 182. 1996. Basionym: *Sclerolobium paraense*

Huber, Bol. Mus. Paraense Hist. Nat. 6: 79. 1910. TYPE: Brazil. Pará: "in silvis paraensibus ad viam ferream inter capitalem et Bragança," *Rodolpho Siquiera Rodrigues s.n. (MG 9642)* (holotype, MG not seen; isotypes, BM!, F!, K!, MO!, US!).

Sclerolobium albiflorum Benoist, Bull. Soc. Bot. France 66: 383. 1919. *Tachigali albiflora* (Benoist) Zarucchi & Herend., Fl. Venez. Guayana 4: 115. 1998. TYPE: French Guiana. *Benoist 1074* (holotype, P!).

Discussion. This species is best recognized by the leaflets with a cuneate, more or less symmetrical base, linear, densely white pubescent petals, and large stipules. Occasionally, small, strongly revolute bracts are present in the inflorescences. Domatia are absent. Stamens are monomorphic. This species is very similar to *Tachigali melanocarpa*, and differences between the two are discussed under that species. There has been some confusion about the type specimens. On the label of the MG holotype (photo at F), the collector is given as Rodolfo S. Rodrigues and the type locality is listed as "Estação agr. Peixe Boi." Isotypes distributed by RB (no. 5620), with the type information retyped, gives the type locality as Peixe Boi, and R. Siqueira as collector. These labels (on specimens in F, K, and US) also give the MG herbarium number (9642). MO has a sheet with an older, handwritten RB label annotated with the number 5620 and citing Rodolpho Siq. Rodrigues as collector. The type locality is still given as Peixe Boi. Dwyer, in his revision of *Sclerolobium* (1957), stated that he had not seen the type of *Sclerolobium paraense*, but cited among the specimens he had seen "Estação de Peixe Boi, *Rodrigues 9642*" (MO) and "Peixe Boi, R.R. between Belem do Para & Bragança, Sigueira Herb. no. 5620" (K, NY, US), all of which I consider isotypes.

Distribution. *Tachigali paraensis* is known from the states of Maranhão and Pará in Brazil, French Guiana, Suriname, and eastern Venezuela (Bolívar, Delta Amacuro).

Selected specimens examined. BRAZIL. **Maranhão:** Mun. Carutapera, *Balee & Ribeiro 2692* (MO). **Pará:** Km 93 Rodovia Belem–Brasilia, *Kuhlmann & Jimbo 42* (IAN, MG, NY, SP). FRENCH GUIANA. **Saül:** La Fumée Mountain Trail, *Mori & Hartley 18460* (MO). SURINAME. Jodensavanne Mapanekreek area, *Elburg LBB 9462* (MO). VENEZUELA. **Bolívar:** al sur de El Dorado, en el drenaje del Río Cuyuni, *Stevermark & Dunsterville 104447* (MO, NY, US). **Delta Amacuro:** este–noreste de El Palmar, *Marcano Berti 216* (INPA, MBM, MO, NY).

39. Tachigali peruviana (Dwyer) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. *Sclerolobium paniculatum* var. *peruvianum* Dwyer, Lloydia 20: 94. 1957. TYPE: Peru. San Martín: Alto Río Huallaga, *Williams 5701* (holotype, F not seen).

Discussion. *Tachigali peruviana* can be recognized by the combination of the sericeous lower surface of the leaflets, the linear, glabrous petals, pedicellate flowers, asymmetrical base of the leaflets, and lack of domatia. Stipules are caducous, and none were seen in this study. It differs from *T. vulgaris* L. F. Gomes da Silva & H. C. Lima in the dense, sericeous indument of the lower surface of the leaflets. *Tachigali subvelutina* (Benth.) Oliveiro-Filho is very similar; it has a denser, semi-erect indument on the leaflets and twigs. This is best seen on the inflorescence axes and rachis of leaves, which are strictly appressed pubescent in *T. peruviana*. Two collections from Humaitá, Amazonas (*Teixeira et al. 199 & 912*), are provisionally placed here; they have larger and wider leaflets, and the flower-bearing branches of the inflorescences are much longer and flexuous. The type of *T. peruviana* has not been seen in this study. *Tachigali subvelutina* has a distinct geographical distribution, a different indument, and occurs in cerrado vegetation and, therefore, should not be confused with *T. peruviana*. However, the differences between the two species are otherwise weak.

Selected specimens examined. BOLIVIA. **Beni:** Km 29 of rd. from Riberalta to Guayaramirim, *Maas et al. 8675* (MO). **Santa Cruz:** Parque Nacional Noel Kempff, *Guillen & Killeen 2710* (MO, NY). BRAZIL. **Amazonas:** Mun. Humaitá, *Teixeira et al. 912* (INPA, MEL, MG, MO). **Rondônia:** Km 5 da rodovia Vilhena–Porto Velho, *Silva & Pinheiro 4112* (INPA, MG, NY). PERU. **Junín:** Chunchuyacu, *Woytkowski 5578* (MO, NY).

40. Tachigali physophora (Huber) Zarucchi & Herend., Fl. Venez. Guayana 4: 116. 1998. Basionym: *Sclerolobium physophorum* Huber, Bol. Mus. Paraense Hist. Nat. 6: 80. 1910. TYPE: Brazil. Amazonas: "ad ripas fl. Japura inferioris," *Ducke s.n. (MG 6751)* (holotype, MG not seen; isotype, BM!).

Discussion. The swollen, cylindrical domatia close to the base of the leaf petiole are characteristic in this species. Very frequently the domatia are longitudinally ribbed. The leaflets are strongly unequal at the base and are sparsely to moderately appressed pubescent on the lower surface. Stipules are usually persistent, 1- to 3-foliose, and 1–2 cm. Petals are linear and glabrous. Stamens are monomorphic. The hypanthium is symmetrical. *Tachigali physophora* is a species of flooded forests. Most collections are from Brazil (Amazonas) with a few collections from adjacent Colombia (Guainía) and Venezuela (Amazonas).

Selected specimens examined. BRAZIL. **Amazonas:** Anavilhanas Island, *Mori et al. 21276* (INPA, MO, NY). COLOMBIA. **Guainía:** Río Inirida, *Garcia-Barriga 20809* (COL, F, GH). VENEZUELA. **Amazonas:** on bank of Río Manapiare betw. San Juan & Morrocoy, *Berry 1604* (MO).

41. Tachigali pimichinensis (R. S. Cowan) Zarucchi & Herend., Fl. Venez. Guayana 4: 120. 1998. Basionym: *Sclerolobium pimichinense* R. S. Cowan, Mem. New York Bot. Gard. 10: 85. 1961. TYPE: Venezuela. Amazonas: Río Guainía, along Cano Pimichin, *Maguire et al. 41830* (holotype, US not seen; isotypes, GH!, MO!, P!).

Discussion. Distinctive in this species are the obtuse to rounded tips of the leaflets. Other characters include the sericeous indument on the lower surface of the leaflets, the occasional presence of small, entire, revolute bracts along the inflorescences, the absence of domatia, and the linear, pilose petals. The hypanthium is symmetrical. Stamens are monomorphic. The leaflets have a symmetrical base.

Tachigali pimichinensis is only known from the type collection.

42. Tachigali plumbea Ducke, Bol. Tecn. Inst. Agron. N. 2: 15. 1944. TYPE: Brazil. Amazonas: Manaus, *A. Ducke 817* (lectotype, designated here, NY not seen; duplicates, IAN!, R!). EPITYPE: *Ducke s.n. (RB 24289)* (epitype, designated here, US!; duplicates, INPA!, K!, P!).

Discussion. Differences between this species and the similar *Tachigali cavipes* are discussed under the latter. *Tachigali plumbea* has sericeous lower surfaces of the leaflets, which have an asymmetrical base, semiterete domatia, dimorphic stamens, and foliose stipules that are often lacking. This species has been reported from the lower Rio Negro and the Rio Madeira.

Ducke (1944) included five collections in his description of *Tachigali plumbea*, four flowering and one sterile. Two collections came from Porto Velho, the other three from around Manaus. Dwyer (1954) cited *Ducke 817*, the sterile collection, as type. He listed two specimens of this collection at NY and US. The lectotype should be chosen from those two, and the NY specimen is here selected as the lectotype. The online image shows as nice a sterile specimen as one can wish for. The specimens from Porto Velho (*Ducke 228* according to the herbarium sheets, *Ducke 288* according to the protologue, and *Ducke s.n. (RB 35419)*) differ in minor aspects from the specimens collected around Manaus (*Ducke 817*, *Ducke 818*, and *Ducke s.n. (RB 24289)*). Dwyer (1954) excluded the Porto Velho specimens from *T. plumbea* and placed them in *T. cavipes*. In this treatment, all five collections are included in *T. plumbea*, while acknowledging that further research may well show that two species are involved. Given this ambiguity, the US sheet of *Ducke s.n. (RB 24289)*, from the same locality as the lectotype, is selected here as epitype. It is a good flowering specimen, displaying domatia, stipules, and both surfaces of the leaflets.

Selected specimen examined. BRAZIL. **Amazonas:** Reserva Florestal Ducke, *Martins & Assunção 91* (MO).

43. Tachigali poeppigiana Tul., Arch. Mus. Hist. Nat. 4: 168. 1844. TYPE: Brazil. Amazonas: Ega, *Poeppig 2837* (holotype, P!; isotype, L!).

Tachigali polyphylla Poepp., Nov. Gen. Sp. Pl. 3: 60. 1845. TYPE: Brazil. Amazonas: Ega, *Poeppig 2837* (holotype, W not seen; isotypes, L!, P!).

Discussion. Distinctive in this species are the many pairs of leaflets (often 10 pairs) and the appressed, grey-brown hairs on the calyx. The hypanthium is asymmetrical. Petals are 2–3 × ca. 1.5 mm. Stamens are monomorphic. Domatia are present but may be slender. Stipules are foliose with one pair of lobes, caducous, and occasionally present in sterile specimens. Bracts are sometimes present in the inflorescences and resemble the stipules but are smaller. The base of the leaflets is strongly asymmetrical. This species can be confused with *Tachigali schultesiana*, but the latter differs in the characters mentioned in the key. Dwyer (1954) used the name *T. polyphylla* for this species, but the name *T. poeppigiana* has priority. *Tachigali poeppigiana* is known from a few collections in Amazonas and one from Rondônia, Brazil. Most collections, but not all, come from riverbanks or flooded forest.

Poeppig (1845) was not aware of Tulasne's publication (Tulasne, 1844). Both Poeppig and Tulasne recognized *Poeppig 2837* as an undescribed species, and both described it independently with the same collection as type (but with different holotypes).

Selected specimens examined. BRAZIL. **Amazonas:** Humaitá, *Krukoff 7233* (GH, MO, US). **Rondônia:** a 13 km de Vilhena, *Vieira et al. 914* (INPA, MO, US).

44. Tachigali prancei (H. S. Irwin & Arroyo) L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 100. 2007. Basionym: *Sclerolobium prancei* H. S. Irwin & Arroyo, Brittonia 26: 268. 1974. TYPE: Brazil. Rondônia: Serra dos Tres Irmãos, N bank of the Rio Madeira, *Prance et al. 5524* (holotype, NY not seen; isotypes, COL!, F!, GH!, INPA!, MG!, R!, RB not seen).

Discussion. *Tachigali prancei* is easily recognized by its large, oblong to ovate-oblong, tomentulose leaf-

lets with a rounded base, tomentulose inflorescences, and linear, pilose petals. Domatia are lacking. Stipules are foliose, flat, ca. 2.5 cm, but rarely present. The apex of the leaflets tapers to an acute or acuminate tip. Floral bracts are about as long as, or shorter than, the buds. Three collections of this species have been seen, all from Rondônia. *Tachigali prancei* can be confused with *T. chrysaloides*, which differs in having an appressed indument on twigs and leaf rachis and in its large, revolute stipules.

Additional specimens examined. BRAZIL. **Rondônia:** Mun. Jaru, *Cid et al. 1987* (MEL, MG, MO); Mun. Ariquemes, *Cid et al. 5058* (MO, NY).

45. Tachigali ptychophysca Benth., Fl. Bras. 15: 229. 1870. TYPE: Brazil. Amazonas: "prope Panure ad Rio Uaupes," *Spruce 2644* (holotype, K!; isotypes, GH!, P!).

Discussion. Although the leaflets of this species are not particularly small (to 11–12 cm long in the GH isotype, to 16 cm long fide Dwyer), they have only two or three pairs of lateral veins and this character alone allows easy identification. Domatia are present and cylindrical. Stamens are dimorphic. The base of the leaflets is symmetrical or nearly so. Bracteoles at the base of the flowers are ca. 3 mm wide, wider than in most other species. Stipules, when present, are foliose with one pair of lobes; lobes are to 1 × 0.6 cm. The few flowering collections seen in this study all have the lower surface of the leaflets densely appressed (somewhat sericeous) and pubescent. *Tachigali ptychophysca* is known from the upper Rio Negro and along the Rio Vaupes in Brazil and Colombia. This species is similar to *T. loretensis*; differences are discussed under the latter.

Selected specimens examined. BRAZIL. **Amazonas:** Taracuá, bacia do alto Rio Negro, *Pires & Silva 7886* (IAN). COLOMBIA. **Amazonas-Vaupes:** Rio Apaporis, Raudal de Jirijirimo, *Schultes & Cabrera 13488* (COL, F, GH, MO).

46. Tachigali pubiflora Benth., J. Bot. (Hooker) 2: 94. 1840. TYPE: Guyana, *Schomburgk 43* (holotype, K!; isotypes, GH!, L!, P!).

Discussion. This species is best recognized by the combination of densely pubescent leaflets, absence of domatia, dense, erect indument on twigs and inflorescences, oblique hypanthium, narrowly foliose stipules, and dimorphic stamens. The base of the leaflets is symmetrical to slightly asymmetrical. The species can be confused with *Tachigali rigida*, which differs in the presence of domatia. *Tachigali pubiflora* is known from Guyana and adjacent Venezuela. Most specimens have a dense indument on the lower surface of the leaflets; however, an occasional specimen (for instance, *Clarke 277* from Guyana) has a sparse indument.

Selected specimens examined. GUYANA. Kaieteur Plateau, forest along Potaro River, *Cowan & Soderstrom 2089* (F, US). VENEZUELA. **Bolívar:** Campamento Ucaima, sureste de Canaima, *Steyermark 106423* (US).

47. Tachigali richardiana Tul., Arch. Mus. Hist. Nat. 4: 166. 1844. TYPE: French Guiana, *Richard Herbarium s.n.* (holotype, P!).

Tachigali sulcata Benoist, Bull. Mus. Hist. Nat. (Paris) 31: 469. 1925. Syn. nov. TYPE: French Guiana. Gourdonville, *Benoist 1574* (holotype, P!).

Tachigali bracteolata Dwyer, Ann. Missouri Bot. Gard. 41: 233. 1954. Syn. nov. TYPE: French Guiana, *Martin s.n.* (holotype, F!).

Discussion. Tachigali richardiana is best recognized by the combination of terete leaf rachises, dimorphic stamens, a basal patch of hairs on the outer surface of the petals, and relatively few lateral veins (4 to 7 pairs per leaflet). It is closely related to *T. alba*, from which it differs in the characters given above and in the slightly larger petals (5–7 mm long vs. 4–5 mm long in *T. alba*). Domatia are lacking, and stipules have not been seen on any of the collections. Occasionally, a collection of *T. richardiana* may not have all the characters mentioned above. For instance, *Sabatier 987* has five pairs of lateral veins and dimorphic stamens, but the petals lack the hairs on the outer surface. *Tachigali barnebyi* is also similar, but differs from *T. richardiana* in its persistent stipules and the gland-dotted lower surface of the leaflets. *Tachigali richardiana* has been reported from terra firme forests in French Guiana and Brazil. Benoist considered *T. sulcata* different from *T. richardiana* in its slightly denser indument and a different shape of bracts. Dwyer (1954) published *T. bracteolata* as a distinct species based on its long bracts. Here, these species are treated as synonyms of *T. richardiana*.

Selected specimens examined. BRAZIL. **Pará:** Estrada do Munguba, *Silva 3075* (IAN). FRENCH GUIANA. RN2 Cayenne–Régina, pk. 79 Camp Hervo, *Sabatier & Prevost 5050* (MO).

48. Tachigali rigida Ducke, Arq. Inst. Biol. Veg. 4(1): 12. 1938. TYPE: Brazil. Amazonas: Igarape Macacuny prope Cucuhy, *Ducke s.n. (RB 35423)* (holotype, RB not seen; isotype, K!).

Tachigali rigida var. *argentata* Ducke, Arq. Inst. Biol. Veg. 4: 12. 1938. TYPE: Brazil. Amazonas: Rio Curicuriari, affl. Rio Negro, *Ducke s.n. (RB 35422)* (holotype, RB!).

Discussion. This species is easily recognized by the combination of the densely appressed pubescent lower surface of the leaflets, the symmetrical base of

the leaflets, the presence of domatia, and the large flowers with dimorphic stamens. The hypanthium is oblique. Stipules are foliose, with one pair of lateral lobes. The characters used by Ducke to recognize variety *argentata* (absence of reddish hairs on lower surface of leaflets, absence of hairs on the upper surface of mature leaflets) are variable, and the variety is here included in synonymy. *Tachigali rigida* resembles *T. pubiflora*; the two species can be distinguished by the presence of domatia in *T. rigida* and their absence in *T. pubiflora*. *Tachigali rigida* is also similar to *T. grandistipulata*, but the latter has smaller flowers with monomorphic stamens, these about as long as the petals. *Tachigali rigida* has stamens much longer than the petals. *Tachigali rigida* is known from the upper Rio Negro in Brazil, from one collection from Vaupes, Colombia, and from Amazonas in Venezuela, frequently growing along rivers in seasonally inundated forest.

Selected specimens examined. BRAZIL. **Amazonas:** entre Camanaus e Curicuriari, *Silva et al. 1649* (INPA). COLOMBIA. **Vaupes:** at confluence of Ríos Guainia and Casiquiare, *Schultes & Lopez 9389* (F, GH, IAN, US). VENEZUELA. **Amazonas:** frequent along Ríos Pacimoni and Yatua, *Maguire et al. 41578* (GH, IAN, MO, US).

49. Tachigali schultesiana Dwyer, Bot. Mus. Leafl. 18: 152. 1958. TYPE: Colombia. Amazonas–Vaupes: Río Apaporis, *Schultes & Cabrera 14045* (holotype, MO!; isotypes, GH!, US!).

Discussion. Characteristic in this species are the many pairs of leaflets (up to 15 pairs), the dense, puberulous, brown indument on inflorescences and flowers, and the nearly symmetrical flowers. Stamens are monomorphic, stipules are foliose but rarely present, and domatia are present and terete. Petals are elliptic, ca. 4 × 2 mm. The base of the leaflets is strongly asymmetrical. The type has flowers and young fruits; the young fruits (15 mm long) are seated on a slender stipe, 5 mm long. *Tachigali poeppigiana* is very similar, but can be distinguished by the differences mentioned in the key. *Tachigali schultesiana* is known from Colombia (Vaupes, Caqueta, Amazonas) and Peru (Loreto).

Selected specimens examined. COLOMBIA. **Amazonas-Vaupes:** Río Apaporis, *Garcia-Barriga 13943* (COL, US). **Caqueta:** Araracuara, *Cardenas et al. 4421* (MO). PERU. **Loreto:** Jenaro Herrera, *Revilla 1220* (MG, MO).

50. Tachigali setifera (Ducke) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Basionym: *Sclerolobium setiferum* Ducke, Arq. Inst. Biol. Veg. 2: 42. 1935. TYPE: Brazil. Amazonas: "silva non inundabilis coliniae Campos Salles prope Manaos," *Ducke s.n. (RB 23329)* (lectotype, designated by Zarucchi & Herendeen in Brako & Zarucchi, 1993: 1254, US!; duplicate, P!).

Discussion. The type has a characteristic, rather dense indument on the lower surface of the leaflets, consisting of appressed, whitish hairs that radiate from a central point in various directions. This indument has not been found on any other species. Stipules are 3-foliose, with the segments revolute and are up to 3 cm long on young shoots. Domatia are lacking. Petals are linear and pilose. The base of the leaflets varies from obtuse to subcordate and is symmetrical. Relationships are with a group of species including *Tachigali rugosa*, *T. prancei*, *T. vasquezii*, and *T. chrysaloides*. These species all differ from *T. setifera* in indument type.

A number of collections that, in all other characters, match *Tachigali setifera* do not have the indument of short, appressed, white hairs. Instead, the lower surface of the leaflets is glabrous or has scattered, short, erect, hairs or a brown scurf. Both forms have a similar geographical distribution: the form with the appressed, white hairs is known from Bolivia, Peru, Ecuador, and Brazil (Amazonas, Mato Grosso, and Rondônia) and one collection from Colombia, and the form lacking these hairs is known from Bolivia, Peru, and Brazil (Acre, Amazonas, and Rondônia) and single collections from Ecuador and Colombia. Apparently, the two forms do not occur together. Eight collections from the Tambopata area in Peru all have the short, white indument, while eight collections from the Bosque Nacional Alexander von Humboldt, Ucayali, Peru, all lack the short, white hairs. Both forms are placed in *T. setifera* largely because of their overlapping distributions and great similarity in all characters except the indument on the lower surface of the leaflets. In the species identification list, specimens with short, white hairs are identified as *T. setifera* s. str., while specimens lacking these hairs are identified as *T. setifera* s.l.

Selected specimens examined of Tachigali setifera *s. str.* BOLIVIA. **Pando:** Manuripi, cerca Puerto America, *Jardim 1117* (MO, NY). BRAZIL. **Amazonas:** Mun. Borba, *Zarucchi 2857* (INPA, MG). **Rondônia:** W bank of Rio Madeira, 2 km below mouth of Rio Abuña, *Prance et al. 6037* (COL, INPA, MG, NY, US). ECUADOR. **Morona Santiago:** 5–10 km E of Logroño, *Little et al. 630* (US). PERU. **Huánuco:** Pachitea, Codo de Pozuzo, *Foster 9295* (MO). **Loreto:** Río Corrientes, upriver from Tnte. Lopez oil camp, *Gentry et al. 18951* (MO). **Madre de Dios:** Tambopata Nature Reserve, *Gentry & Jaramillo 57661* (MO).

Selected specimens examined of Tachigali setifera *s.l.* BOLIVIA. **Beni:** Prov. Vaca Diez, *Boom 4394* (MO, NY). **Santa Cruz:** Parque Nacional Noel Kempff, *Arroyo et al. 644* (MO). BRAZIL. **Amazonas:** Manaus, Reserva Florestal Ducke, *Aluisio 28* (INPA). **Mato Grosso:** margen esquerda de Rio Juruena, *Rosa & Santos 2074* (MO, NY). **Rondônia:** Mun.

Costa Marques, *Cid Ferreira 8736* (INPA, MEL, MO). ECUADOR. **Morona-Santiago:** 56 km SE de Taisha, *Ortega U. 214* (US). PERU. **Amazonas:** Quebrada chichijam entsa, *Ancuash 275* (MO). **Huánaco:** Tingo Maria, *Burgos 11* (US). **Madre de Dios:** Km 15 de carretera Iberia–Iñapari, *Ruiz C. 27* (MO). **Ucayali:** Aguaytia–Pampa el Sacramento, *Gutierrez R. 43* (F, NY, US).

51. Tachigali tinctoria (Benth.) Zarucchi & Herend., Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. Basionym: *Sclerolobium tinctorium* Benth., Hooker's J. Bot. Kew Garden Misc. 2: 236. 1850. TYPE: Brazil. Pará: prope Caripi, *Spruce s.n.* (holotype, K!; isotype, P!).

Sclerolobium reticulosum Dwyer, Lloydia 20: 98. 1957. *Tachigali reticulosa* (Dwyer) Zarucchi & Herend., Fl. Venez. Guayana 4: 120. 1998. Syn. nov. TYPE. Brazil. Amazonas: Humayta. *Krukoff 7188* (holotype, MO!; isotypes, BM!, GH!, K!).

Discussion. This species is best recognized by the absence of domatia and the sparsely appressed pubescent to glabrous, oblong leaflets with an asymmetrical base. Petals are linear and glabrous. Stipules, when present, are pectinate and appressed pubescent. It is very similar to *Tachigali vulgaris*, the main difference being the obviously pedicellate flowers in *T. vulgaris* versus sessile or very shortly pedicellate flowers in *T. tinctoria*. Most collections of this species are from the Brazilian state of Pará, with a few collections from Amazonas and Venezuela. No differences between the specimens placed by Dwyer (1957a) in his *Sclerolobium reticulosum* and specimens of *T. tinctoria* have been found in this study. *Sclerolobium reticulosum* is consequently placed in synonymy under *T. tinctoria*.

Selected specimens examined. BRAZIL. **Amazonas:** Rio Negro, Padauiry, *Froes 22718* (COL, US). **Pará:** Mun. Almeirim, Mt. Dourado, *Pires & Silva 1376* (INPA, MG, NY). **Rondônia:** Mun. Costa Marques, 123 km de Costa marques, *Cid Ferreira 8672* (INPA, MEL, MO). **Roraima:** Caracarai, *Ducke 1595* (F, MG, NY). VENEZUELA. **Amazonas:** alrededores de Puerto Ayacucho, *Huber 4766* (NY, US).

52. Tachigali vaupesiana van der Werff, sp. nov. TYPE: Colombia. Vaupes: Abiu Igarape, E of Mitu, 20 Nov. 1945, *P. Allen 3366* (holotype, MO!; isotypes, GH!, NY!). Figure 13.

Sclerolobium odoratissimum var. *latifolium* Dwyer, Lloydia 20: 83. 1957. TYPE: Brazil. Amazonas: São Paulo de Olivença, *Krukoff 8988* (holotype, MO!; isotypes, F!, GH!, K!, US!).

Tachigali chrysophyllae (Poepp.) Zarucchi & Herend. similis, sed ab ea foliolis basi aequilateralibus et stipulis integris margine revolutis (nec pectinatis) recedit.

Tree, to 35 m; twigs minutely appressed pubescent, becoming glabrous with age. Leaves 15–25 cm, with 2 to 5 pairs of leaflets; leaf petiole with a swollen domatium 1.5–2 cm from the base, this 2.5–4 cm, the first pair of leaflets attached just above the domatium; leaf stalk finely appressed, grey pubescent; leaflets elliptic, 7–15 × 3–6 cm, the base obtuse, symmetrical or nearly so, the tip acute, the upper surface glabrous, lower surface densely appressed, ± sericeous, pubescent, petiolules 3–5 mm, with a similar indument as the leaf stalk; stipules small, 6–9 mm, with a short (to 3 mm long) stalk, entire, the margin revolute, infrequently present; bracts similar to the stipules occur in the inflorescences. Inflorescences to 25 cm, paniculate, finely and densely appressed pubescent, the flower-bearing branchlets slender, flexuous, to 12 cm long. Flowers with a short (ca. 1 mm) pedicel, densely grey pubescent; hypanthium symmetrical, ca. 1 mm; sepals elliptic, ca. 2 mm; petals ca. 2 mm, linear, densely yellow pubescent; stamens monomorphic, ca. 2.5 mm, with some yellow hairs near the base, the distal 2/3 glabrous; ovary ca. 2 mm, moderately pubescent with stiff, brown hairs, centrally attached in the hypanthium. Fruits not seen.

Discussion. *Tachigali vaupesiana* is only known from three collections: the type, a second collection from São Paulo de Olivença, and a third from São Pedro on the Rio Vaupes, both in Amazonas, Brazil. The type specimens had been previously annotated as *T. chrysophylla* or *Sclerolobium* aff. *chrysophyllum*. *Tachigali vaupesiana* differs notably from that species in the symmetrical base of the leaflets and in its small, entire stipules with a revolute margin. The stipules are inconspicuous; similar bracts are found in the inflorescences. They are present in all four duplicates of the type collection. Another difference between the new species and *T. chrysophylla* is in the position of the domatia: in *T. vaupesiana* the domatia occur below the first pair of leaflets, while in *T. chrysophylla* the domatia often occur distal to the first pair of leaflets. Similar small, entire bracts are also present in *T. eriopetala*, and this species is here considered to be very similar (leaf shape, indument) to *T. vaupesiana*, the main difference being the absence of domatia in *T. eriopetala*. All collections of *T. eriopetala* seen in this study are from the Manaus region or from Pará and none have domatia. Thus, *T. eriopetala* is here considered as the closest relative of *T. vaupesiana*, and *T. vaupesiana* is recognized as a distinct species based on the presence of domatia and its distribution. *Sclerolobium odoratissimum* var. *latifolium* is placed in synonymy of *T. vaupesiana*. Its type, *Krukoff 8988*, agrees well in leaf and indument characters with *T. vaupesiana*, and it has the same domatia. Stipules are lacking in the two duplicates of *Krukoff 8988* seen in this study; this is probably due to

Figure 13. *Tachigali vaupesiana* (holotype).

these specimens being in young fruit by which time the stipules have commonly fallen off. Because of the importance of floral characters, a flowering collection is chosen as the type of *T. vaupesiana*. *Sclerolobium odoratissimum* var. *latifolium* is not raised to species rank; otherwise, a collection with young fruits would be the type collection.

IUCN Red List category. This species is known only from three collections and has been given the listing of Data Deficient (DD) (IUCN, 2001).

Paratype. BRAZIL. **Amazonas:** Rio Waupes, Mun. São Gabriel da Cachoeira, *Damião 2998* (INPA).

53. Tachigali venusta Dwyer, Ann. Missouri Bot. Gard. 41: 235. 1954. TYPE: Brazil. Pará: Tapajoz, Boa Vista, *Capucho 418* (holotype, F not seen).

Discussion. *Tachigali venusta* is very similar to *T. plumbea* and *T. cavipes* in its sericeous pubescence, semiterete domatia, dimorphic stamens, and asymmetrical base of the leaflets. It can be separated from the other two species by its stipules with filiform segments and the nearly sessile flowers. Stipules are not always present, but in that case the nearly sessile flowers allow identification. The upper surface of the leaflets is almost as densely pubescent as the lower surface, while in *T. plumbea* and *T. cavipes* the upper surface is often less densely pubescent. The type of *T. venusta* has not been seen, and the species concept is based on the paratype *Ducke 1989*. *Tachigali venusta* has been reported from terra firme forest on sandy soil in the Brazilian states of Amazonas, Pará, and Rondônia.

Selected specimens examined. BRAZIL. **Amazonas:** Manaus, Reserva Florestal Ducke, *Rodrigues 5431* (F, INPA, US). **Pará:** 27 km NE of Itaituba, *Prance et al. 25804* (IAN, MG, MO, US). **Rondônia:** Mun. Porto Velho, *Cid Ferreira 7451* (INPA, MEL, MO).

54. Tachigali vulgaris L. F. Gomes da Silva & H. C. Lima, Rodriguésia 58: 400. 2007. Replaced synonym: *Sclerolobium paniculatum* Vogel, Linnaea 11: 397. 1837, non *Tachigali paniculata* Aubl., 1775. TYPE: Brazil. Mato Grosso: in regione Cujaba, *Manso & Lhotzky s.n.* (holotype, B†; isotype, MO!).

Discussion. *Tachigali vulgaris* is a common and widespread species, best recognized by moderately dense, appressed indument on the lower surface of the leaflets, caducous stipules and domatia, the glabrous, linear petals, and the unequal base of the leaflets. It is very similar to *T. tinctoria*, which differs in its sessile flowers; flowers of *T. vulgaris* are distinctly pedicellate. The type of this species is from a collection by Manso and Lhotzky from the Cuiabá region in Mato Grosso (Vogel, 1837). Bentham (1870) recognized three varieties of *Sclerolobium paniculatum*: variety *paniculatum*, variety *rubiginosum* (Tul.) Benth., and variety *subvelutinum* Benth. The latter two varieties have not yet been reported from the Amazon forests. Dwyer (1957a) published an additional variety, variety *peruvianum* Dwyer, from Peru. These varieties of *S. paniculatum* have now been raised to species, as *T. peruviana*, *T. rubiginosa* (Tul.) Oliveira-Filho, and *T. subvelutina*. Specimens of *T. vulgaris* are known from Pará, Mato Grosso, Rondônia, and a few collections from Amazonas and Bolivia. Many specimens from Pará differ in indument from specimens from Mato Grosso and Rondônia; the indument on twigs is less dense and predominantly erect, and the leaflets also have a partly erect indument. However, there are several intermediate specimens, and *T. vulgaris* is here accepted as a species with a somewhat variable indument, occurring in dry forest (campos, campinas) along the southern margin of the Amazon rainforest from Pará to Bolivia. Both Lemee (1952) and Oliveira Filho (2006) mention that *S. paniculatum* and *S. melinonii* are probably conspecific. Here, *S. melinonii* (as *T. melinonii*) and *T. vulgaris* are considered as distinct species, largely based on the presence of the characteristic small, stellate hairs on *T. melinonii*.

Incompletely Known Species

Following is a list of validly published species of which no specimens have been seen or which are only known from fruiting collections. Also included is a likely undescribed species.

Sclerolobium herthae Harms, Notizbl. Bot. Gart. Berlin-Dahlem 15: 46. 1940. TYPE: Ecuador. Pastaza: Mera, *Schultze-Rhonhof 2922* (holotype, B).

The type and only collection was presumably destroyed in the Berlin herbarium during World War II.

Tachigali multijuga Benth., Fl. Bras. 15(2): 229. 1870. TYPE: Brazil. "in sylvis Capoeiras pr. Uananuca ad Rio Negro," *Spruce 2022* (holotype, K not seen).

Bentham also mentioned very similar specimens collected near Rio de Janeiro. Ducke (1949) stated that he had not seen specimens from the upper Rio Negro, the type locality, but that it was frequent around Rio de Janeiro. Dwyer (1954) cited, in addition to the Spruce collection, six collections from southern Brazil. Bentham compared it with *Tachigali poeppigiana*, from which it differs in having much larger flowers. Specimens identified as *T. multijuga* from

southern Brazil indeed have very large flowers, but no specimens like it (characterized by large flowers and 9 to 15 pairs of leaflets) have been seen from the Rio Negro in Amazonas, and it is not certain if *T. multijuga* occurs in Amazonas. The specimens from southern Brazil formerly placed in *T. multijuga* are now included in *T. paratyensis* (Vell.) H. C. Lima.

Tachigali vasquezii Pipoly, Sida 16: 408. 1995. TYPE: Peru. Amazonas: Valle del Río Santiago, Quebrada Caterpiza, *Huashikat 1910* (holotype, MO!).

The holotype is a fruiting specimen. The lower surface of the leaflets is densely tomentulose, leaflets are oblong, with a rounded base, tertiary venation is raised on the lower surface, and the hypanthium is symmetrical. Domatia and stipules are absent. A second collection, *Huashikat 1408* (MO), is also in fruit. Both collections were made 2–3 km from the community of Caterpiza. The sterile paratypes of *Tachigali vasquezii* are here considered to be *T. setifera* s. str. The differences in indument between *T. setifera* and *T. vasquezii* are striking, and no intermediates have been seen. Nevertheless, I consider the two species closely related, based on the oblong leaflets with a rounded, symmetrical base.

Tachigali sp. 1

Tall trees (height not given), resembling *Tachigali chrysophylla* in the sericeous lower surface of the leaflets and shape and position of the domatia (3–5 cm from base of leaf stalk). It differs from that species in having pilose (not glabrous) petals and a symmetrical base of the leaflets that is recurved. Leaflets are 9–20 cm, elliptic to oblong. One sterile collection has stipules; these are ca. 1.5 cm, pinnately divided, the segments linear and distally widened in a small pouch (Fig. 1G). No other species from northern South America possesses such stipules. The hypanthium is symmetrical. The NY specimen of *Pires 3696* has old flowers in a pocket. This is very likely an undescribed species, but because good flowers are lacking, it is not described here.

Specimens examined. BRAZIL. **Amazonas:** 400 km E of Humaitá, st., *F. Rickson B-44A-85* (MO). **Border area of Amazonas, Pará, and Mato Grosso:** Boca do Rio Juruena, old flowers, *Pires 3696* (COL, IAN, INPA, NY); Rio São Manoel, limite Pará–Mato Grosso, fallen fruits and leaflets, *Pires 3828* (IAN).

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THE TROPICAL FLORA OF SOUTHERN YUNNAN, CHINA, AND ITS BIOGEOGRAPHIC AFFINITIES[1]

Zhu Hua[2]

ABSTRACT

The flora of Xishuangbanna in southern Yunnan, southwestern China, consists of 3340 native seed plant species belonging to 1176 genera and 182 families. Tropical floristic elements at the generic level form a major contribution (78.3%) to the total flora of southern Yunnan, of which the dominant geographic elements are those of tropical Asian distribution. The tropical Asian flora of Xishuangbanna is similar in composition to other tropical floras from Yunnan, especially in the families with the most species richness. These regional floras have similarities of more than 89% at the family level and more than 76% at the generic level, but share only 43%–50% similarity at the specific level. Comparison with mainland Southeast Asia (Thailand) and Malesia (Malay Peninsula) floras reveals that most of the dominant families from southern Yunnan are also dominant in mainland Southeast Asian and Malesian floras. The floristic similarities between the flora of southern Yunnan and those of tropical Asia are more than 80% at the family level and more than 64% at the generic level. This suggests that the tropical flora of southern Yunnan has a close affinity with tropical Asian flora and supports the idea that the flora of southern Yunnan, together with mainland Southeast Asian flora, belongs to the Indo-Malaysian floristic subkingdom of the Paleotropical kingdom as suggested by Takhtajan, or the Malaysian subkingdom of the Paleotropical kingdom as suggested by Wu and Wu. However, situated at the northern margin of tropical Asia, the flora of southern Yunnan comprises less strictly tropical elements compared to Malaysian flora and, consequently, represents only a marginal type of Indo-Malaysian flora. The tropical flora of southern Yunnan is supposed to be derived from tropical Asian flora with the formation of the eastern monsoon climate after the Tertiary.

Key words: Biogeographic affinity, southern Yunnan, floristic composition.

The tropical area of southern China is located at the northern edge of tropical Asia and is composed of the extreme southeastern part of Xizang (Tibet) among the lower valleys of the southern Himalayas, southern Yunnan, southwestern Guangxi, southern Taiwan, and Hainan separately. The largest tropical area still covered by forests is in southern Yunnan, the most southwestern region of China, which is also a key area in biogeography and a hotspot for biodiversity (Myers, 1998). This is a mountainous region at the northern margin of mainland Southeast Asia, with a monsoon climate and a slightly lower annual mean temperature (ca. 21°C) and lower annual precipitation (average, 1500 mm) in lowlands and valleys compared to the main tropical rainforest areas of the world, e.g., Malaysia (Richards, 1996). The tropical forest in southern Yunnan is therefore intermediate between classic tropical rainforests and monsoon forests as defined by Schimper (1903), or a type of subtropical rainforest, which differs in various aspects from the true tropical rainforests described by Richards (1952).

After the Chinese-Russian expedition to remote areas of southwestern China, including southern Yunnan, in the late 1950s, papers on the tropical rainforest vegetation (Fedorov, 1958; Wang, 1961) and tropical flora (Fedorov, 1957; Wu, 1965) of this part of China were published. It was basically accepted that biogeographically real tropical rainforests exist in southwestern China, but these were considered to be a different type from those in Indo-Malaysia because of the lack of representatives from Dipterocarpaceae, which dominate the rainforests of tropical Southeast Asia.

Botanical interest was rekindled in the 1970s by the discovery of a dipterocarp forest in southern Yunnan, thus the Indo-Malaysian affinity of the tropical flora of Yunnan was reconsidered. Further results from biogeographic and ecological studies of the vegetation and flora of tropical southern Yunnan revealed that it does in fact comprise a part of the Indo-Malaysian flora (Zhu, 1992, 1993a, b, 1994, 1997, 2004; Zhu et al., 1998a, b, 2001, 2003; Zhu &

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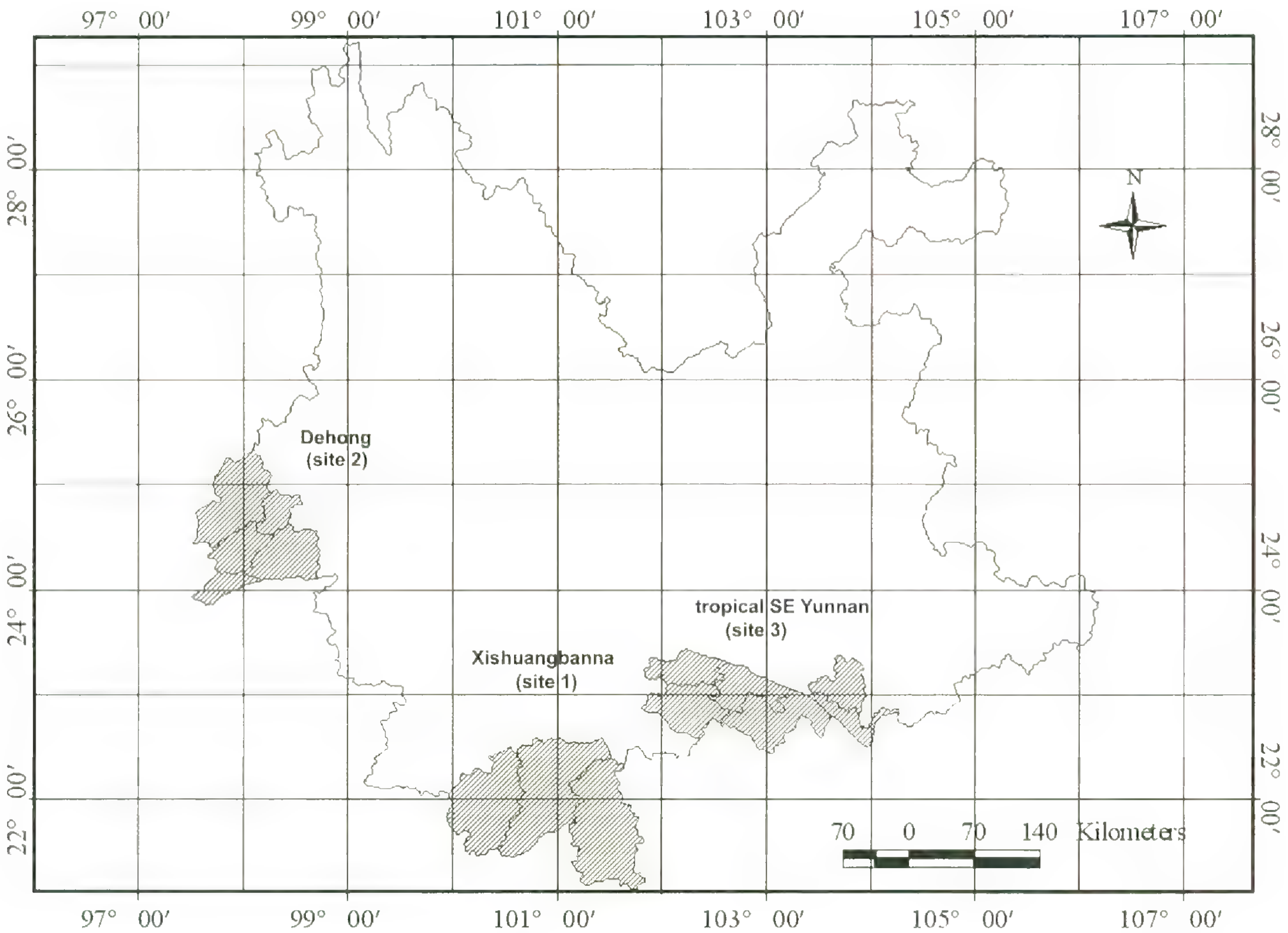

Figure 1. Map showing the locations of the tropical areas in southern Yunnan. Site 1: Xishuangbanna administrative region in south Yunnan; site 2: Dehong administrative region in southwestern Yunnan; site 3: tropical southeastern Yunnan.

Roos, 2004). Also, T. C. Whitmore, from his visit to southern Yunnan, observed that the birds in the tropical rainforest there sang the same songs as the birds in the tropical rainforest of Malaysia (Whitmore, 1982), and he confirmed that there is true evergreen rainforest present in the southern fringe of China (Whitmore, 1984).

To better understand the affinities of southern Yunnan's flora, the floristic composition was concisely enumerated, and its geographic elements were analyzed at family and generic levels. Also, the floristic similarities between southern, southeastern, and southwestern Yunnan (Fig. 1, sites 1–3), as well as between mainland Southeast Asia (Thailand) and Malesia (Malay Peninsula), were compared using revised floristic inventories and checklists.

Geography

Topography

Xishuangbanna in southern Yunnan lies between 21°09′N and 22°36′N, 99°58′E and 101°50′E (Fig. 1). The region occupies an area of 19,690 km^2. It borders Burma and Laos and has a mountainous topography with mountain ridges running in a north-south direction, decreasing in elevation southward. Its elevation ranges from 480 m at the lowest valley bottom in the south (Mekong River) to 2429.5 m at the highest mountain summit in the north. The Mekong River runs through the region from the northwest to the southeast (Xu et al., 1987).

Climate

The region has a typical monsoon climate. The annual mean temperature varies from 21.7 C at an elevation of 550 m to 15.1 C at 1979 m, and the 20 C isotherm is equal to the 850 m elevation isoline. The hottest month is June, with a mean temperature of 17.9°C at 1979 m and 25.3°C at 550 m, while the coldest month is January, with a mean temperature ranging from 8.8°C–15.6°C. Annual precipitation increases from 1193 mm at Mengyang in the central part of the region at 740 m to 2491 mm at the summit of Nangongshan in the southern part at 1979 m. More than 80% of the precipitation falls during the rainy season from May to October. In areas of lower hills and valleys covered by tropical rainforests, the annual mean temperature is about 21°C and the annual cumulative temperature (the sum of daily temperature means > 10 C) is 7600 C–7800 C. Annual precip-

itation is more than 1500 mm, relative humidity is about 80%, and frost has never been recorded (Xu et al., 1987).

Dense fog is frequent throughout the dry season on the lower hills and in the valleys, averaging 146 foggy days per year and 1 mm precipitation per foggy day in Mengla Xian in southern Xishuangbanna. The fog is thought to compensate for the insufficient precipitation so that a tropical moist climate can form locally despite relatively low annual precipitation (Zhu, 1997).

SOIL

There are three main soil types in the region. Lateritic soils developed from siliceous rocks such as granite, and gneiss occurs at 600–1000 m with a deep solum, but thin humus horizon. Lateritic red soils derived from sandstone substrate occur in areas above 1000 m. Limestone hill soils are derived from a hard limestone substrate of Permian origin with a pH of 6.75. The tropical rainforest in southern Yunnan occurs mainly on lateritic soils with pH values of 4.5–5.5.

GEOLOGICAL HISTORY

Southern Yunnan is at the junction of the Indian and Burmese plates of Gondwana and the Eurasian plate of Laurasia (Audley-Charles, 1987; Hall, 1998). Before the Mesozoic, the area was part of the Tethys margin, and later some fragments from Gondwana were combined. Since the Tertiary, the region had gone through several stages of rising and descending with the intermittent uplift of the Himalayas, and gradually formed the modern topography in the mid-Tertiary (Shi et al., 1998, 1999).

During the late Cretaceous, the region had a hot, dry climate, based on fossil records from Mengla in which a relatively high proportion of *Ulmipollenites* and *Ephedripites* were found (Sun, 1979). From the Paleocene to the Eocene, the region went through a rising stage with the uplift of the Himalayas and was influenced by a dry climate with a high deposition of salt minerals. From the Miocene to Pliocene, the region descended and formed a series of basins with a wet and warm climate. Since the Quaternary, the region has again experienced rapid uplift, associated with alternative climatic changes of wet and dry periods (Liu et al., 1986).

Paleobotanical data from the Tertiary in southern Yunnan (Xishuangbanna) are extremely insufficient. Available information from neighboring regions suggests that southern Yunnan could be a subtropical evergreen broad-leaved forest characterized by Fagaceae species during this period (Song et al., 1976, 1983; Song, 1984; Wang, 1996; Penny, 2001; Mehrotra et al., 2005).

The present tropical rainforest in southern Yunnan is at the elevational and latitudinal limits of tropical rainforests in the Northern Hemisphere. It is believed that the tropical moist climate in southern Yunnan did not form until the Himalayas uplifted to a certain elevation after the late Tertiary (Zhu, 1997). Thus, the tropical rainforests in the region were developed after the later Tertiary. The fundamental topography and climate of the region have been strongly affected by the uplift of the Himalayas and the formation of the eastern monsoon climate (Shi et al., 1998, 1999).

METHODS

Based on identifications of ca. 60,000 specimens from southern Yunnan in HITBC, acquired during more than 40 years of intermittent field collections, the former *List of Plants in Xishuangbanna* (Li, 1996) was revised and updated, and 3340 native species of 1176 genera and 182 families of seed plants have been recognized (see Appendix 1). Circumscription of families and species nomenclature follows w³TROPICOS of the Missouri Botanical Garden (<http://www.tropicos.org>). Floristic and geographic attributes of the flora of southern Yunnan were analyzed. Patterns of seed plant distribution of the flora were quantified at the family and generic levels based on C. Y. Wu's documentations (Wu, 1991; Wu et al., 2003). The inventory data of the floras of southwestern Yunnan from the Dehong administrative region (Zhu et al., 2004) and southeastern Yunnan (Shui, 2003) (see Fig. 1, sites 2 and 3, respectively) were compared to demonstrate the floristic variation in tropical Yunnan. The revised checklist of the vascular plants of Malay Peninsula (Turner, 1995) and the checklist of Thai plants (Smitinand, 2001) were also used to compare the floristic affinity between the tropical flora of southern Yunnan (Fig. 1, site 1) and that of mainland Southeast Asia and Malaysia.

FLORISTIC COMPOSITION

A total of 3340 native species of 1176 genera and 182 families of seed plants were recognized from Xishuangbanna in southern Yunnan. The families with highest species richness include Orchidaceae (94 genera/328 species), Fabaceae (65/211), Rubiaceae (45/142), Poaceae (63/132), Euphorbiaceae (36/117), Asteraceae (59/106), Moraceae (7/73), Lauraceae (14/70), Urticaceae (14/72), Zingiberaceae (17/72), Acanthaceae (32/68), Lamiaceae (28/61), Asclepiadaceae (26/58), and Apocynaceae (23/51) (Table 1).

Table 1. The flora of southern Yunnan (Xishuangbanna) by family. Only native species in southern Yunnan (Xishuangbanna) are included. Family size is from the Missouri Botanical Garden's w³TROPICOS (<http://www.tropicos.org>).

	No. of genera	No. of species		No. of genera	No. of species
Families with >100 spp.					
Orchidaceae	94	328	Poaceae	63	132
Fabaceae	65	211	Euphorbiaceae	36	117
Rubiaceae	45	142	Asteraceae	59	106
Families with 51–100 spp.					
Moraceae	7	73	Acanthaceae	32	68
Lauraceae	14	70	Lamiaceae	28	61
Urticaceae	14	72	Asclepiadaceae	26	58
Zingiberaceae	17	72	Apocynaceae	23	51
Families with 21–50 spp.					
Annonaceae	15	49	Scrophulariaceae	15	33
Verbenaceae	11	48	Sterculiaceae	12	33
Cucurbitaceae	17	46	Commelinaceae	10	31
Rosaceae	18	46	Menispermaceae	14	30
Vitaceae	7	45	Gesneriaceae	13	29
Fagaceae	5	44	Myrsinaceae	4	28
Araceae	17	43	Melastomataceae	9	27
Arecaceae	8	40	Araliaceae	10	26
Cyperaceae	14	40	Rhamnaceae	9	26
Rutaceae	14	40	Solanaceae	5	25
Meliaceae	12	37	Myrtaceae	2	23
Convolvulaceae	13	36	Oleaceae	7	23
Liliaceae	19	35	Dioscoreaceae	1	22
Malvaceae	10	34	Theaceae	9	22
Piperaceae	3	33			
Families with 6–20 spp.					
Polygalaceae	4	20	Bignoniaceae	7	10
Polygonaceae	4	19	Lythraceae	4	10
Apiaceae	8	18	Ulmaceae	5	10
Boraginaceae	9	18	Myristicaceae	3	9
Elaeocarpaceae	2	18	Primulaceae	1	9
Loranthaceae	6	18	Symplocaceae	1	9
Anacardiaceae	12	18	Violaceae	1	9
Campanulaceae	9	17	Actinidiaceae	2	8
Tiliaceae	5	17	Burseraceae	3	8
Celastraceae	4	16	Proteaceae	2	8
Flacourtiaceae	6	16	Sabiaceae	2	8
Loganiaceae	6	15	Sapotaceae	3	8
Smilacaceae	1	15	Balsaminaceae	1	7
Caprifoliaceae	5	14	Caryophyllaceae	6	7
Capparaceae	4	14	Hernandiaceae	1	7
Ranunculaceae	4	14	Juglandaceae	3	7
Amaranthaceae	7	13	Lentibulariaceae	1	7
Clusiaceae	5	13	Styracaceae	4	7
Ericaceae	4	13	Viscaceae	2	7
Magnoliaceae	8	13	Aristolochiaceae	1	6
Sapindaceae	11	13	Ebenaceae	1	6
Aquifoliaceae	1	12	Malpighiaceae	2	6
Icacinaceae	8	12	Musaceae	2	6
Begoniaceae	1	11	Onagraceae	2	6
Combretaceae	4	11	Passifloraceae	2	6
Cornaceae	4	11	Pittosporaceae	1	6
Hippocrateaceae	3	11	Santalaceae	5	6

Table 1. Continued.

	No. of genera	No. of species		No. of genera	No. of species
Families with 1–5 spp.					
Brassicaceae	3	5	Podostemaceae	2	2
Elaeagnaceae	1	5	Portulacaceae	2	2
Gentianaceae	5	5	Potamogetonaceae	1	2
Hydrocharitaceae	4	5	Rhizophoraceae	2	2
Oxalidaceae	3	5	Saururaceae	2	2
Simaroubaceae	3	5	Stemonaceae	1	2
Staphyleaceae	2	5	Thymelaeaceae	2	2
Balanophoraceae	1	4	Xyridaceae	1	2
Betulaceae	3	4	Berberidaceae	1	1
Connaraceae	4	4	Cephalotaxaceae	1	1
Dilleniaceae	2	4	Ceratophyllaceae	1	1
Iridaceae	2	4	Crypteroniaceae	1	1
Lemnaceae	3	4	Cupressaceae	1	1
Nyssaceae	2	4	Datiscaceae	1	1
Podocarpaceae	3	4	Dichapetalaceae	1	1
Saxifragaceae	3	4	Droseraceae	1	1
Aizoaceae	2	3	Erythroxylaceae	1	1
Alismataceae	2	3	Geraniaceae	1	1
Amaryllidaceae	2	3	Grossulariaceae	1	1
Burmanniaceae	1	3	Hydrangeaceae	1	1
Chloranthaceae	2	3	Hydrophyllaceae	1	1
Cycadaceae	1	3	Juncaceae	1	1
Gnetaceae	1	3	Lecythidaceae	1	1
Marantaceae	3	3	Menyanthaceae	1	1
Olacaceae	3	3	Myricaceae	1	1
Plantaginaceae	1	3	Najadaceae	1	1
Pontederiaceae	1	3	Nyctaginaceae	1	1
Aceraceae	1	2	Orobanchaceae	1	1
Bombacaceae	1	2	Pandanaceae	1	1
Butomaceae	2	2	Papaveraceae	1	1
Buxaceae	2	2	Pinaceae	1	1
Chenopodiaceae	2	2	Rafflesiaceae	1	1
Crassulaceae	2	2	Salicaceae	1	1
Daphniphyllaceae	2	2	Sonneratiaceae	1	1
Dipterocarpaceae	2	2	Sparganiaceae	1	1
Elatinaceae	2	2	Sphenocleaceae	1	1
Eriocaulaceae	1	2	Stachyuraceae	1	1
Fumariaceae	1	2	Stylidiaceae	1	1
Hamamelidaceae	2	2	Taccaceae	1	1
Lardizabalaceae	1	2	Trapaceae	1	1
Linaceae	2	2	Valerianaceae	1	1
Nymphaeaceae	2	2	Zygophyllaceae	1	1
Opiliaceae	2	2			

Total: 182 families, 1176 genera, and 3340 species including infraspecific taxa

The family Orchidaceae has higher species richness in Xishuangbanna than in Laos and Cambodia (Chen & Tsi, 1996).

Some families have only a small number of species, but are very abundant in the tropical forests of southern Yunnan, such as Sapindaceae (11 genera/13 species), Anacardiaceae (12/18), Burseraceae (3/8), Elaeocarpaceae (2/18), Ebenaceae (1/6), Combretaceae (4/11), Ulmaceae (5/10), and Myrtaceae (2/23). The families Dipterocarpaceae (2/2), Datiscaceae (1/1), Myristicaceae (3/9), Clusiaceae (5/13), Icacinaceae (8/12), Linaceae (2/2), and Sapotaceae (3/8) have even fewer species, but are also very abundant in some forest types.

At the generic level, *Ficus* L. has the most taxa (58 species including infraspecific taxa). Other genera

Table 2. Geographic elements of seed plants at the family level from the flora of southern Yunnan (Xishuangbanna).

Geographic elements	No. of families (%)
1. Cosmopolitan	52 (28.57)
2. Pantropic	77 (42.31)
3. Tropical Asia and tropical America disjunctions	10 (5.49)
4. Old World Tropic	4 (2.20)
5. Tropical Asia to tropical Australia	5 (2.75)
6. Tropical Asia	3 (1.65)
7. North temperate	24 (13.19)
8. East Asia and North America disjunctions	3 (1.65)
9. Old World temperate	1 (0.55)
10. East Asia	3 (1.65)
Total	182 (100.00)

Table 3. Geographic elements of seed plants at the generic level from the flora of southern Yunnan (Xishuangbanna).

Geographic elements at the generic level	No. of genera (%)
1. Cosmopolitan	59 (5.02)
2. Pantropic	251 (21.34)
3. Tropical Asia and tropical America disjunctions	30 (2.55)
4. Old World Tropics	112 (9.52)
5. Tropical Asia to tropical Australia	76 (6.46)
6. Tropical Asia to tropical Africa	96 (8.16)
7. Tropical Asia	355 (30.19)
8. North temperate	60 (5.10)
9. East Asia and North America disjunctions	32 (2.72)
10. Old World temperate	24 (2.04)
11. Temperate Asia	5 (0.43)
12. Mediterranean region, western to central Asia	2 (0.17)
13. Central Asia	1 (0.09)
14. East Asia	62 (5.27)
15. Endemic to China	11 (0.94)
Total	1176 (100)

with high species richness include *Dendrobium* Sw. (43 species), *Bulbophyllum* Thouars (40), *Piper* L. (27), *Calamus* L. (24), *Dioscorea* L. (22), *Syzygium* P. Browne ex Gaertn. (22), *Eria* Lindl. (22), *Litsea* Lam. (21), *Pilea* Lindl. (17), *Lithocarpus* Blume (16), *Millettia* Wight & Arn. (16), *Castanopsis* (D. Don) Spach (16), *Tetrastigma* (Miq.) Planch. (16), *Elaeocarpus* L. (16), *Elatostema* J. R. Forst. & G. Forst. (14), *Amomum* Roxb. (14), *Clerodendrum* L. (14), *Ardisia* Sw. (10), *Lasianthus* Jack (12), *Dysoxylum* Blume (10), and *Fissistigma* Griff. (9). *Pometia* J. R. Forst. & G. Forst., *Terminalia* L., *Antiaris* Lesch., *Gironniera* Gaudich., *Pouteria* Aubl., *Pterospermum* Schreb., and *Tetrameles* R. Br. have fewer species, but are highly abundant in the dominant tree layer of the tropical rainforests. *Lasiococca* Hook. f., *Garcinia* L., *Mitrephora* Hook. f. & Thomson, *Alphonsea* Hook. f. & Thomson, *Cleidion* Blume, *Sumbaviopsis* J. J. Sm., *Trigonostemon* Blume, and *Pittosporopsis* Craib are abundant in the lower tree layer (Zhu et al., 1998a; Zhu, 2006). In the tropical montane rainforest of southern Yunnan, *Castanopsis*, *Lithocarpus*, *Machilus* Nees, *Litsea*, *Phoebe* Nees, *Anneslea* Wall., and *Schima* Reinw. ex Blume are the most abundant genera (Zhu et al., 2006).

Geographic Elements

Geographic elements at the family level

In the flora of southern Yunnan, families of tropical distribution contribute 54.4% of the total flora (Table 2). These families include those of pantropic distribution (contributing to 42.31% of the total flora), such as Acanthaceae, Anacardiaceae, Annonaceae, Apocynaceae, Araceae, Arecaceae, Burseraceae, Clusiaceae, Combretaceae, Myristicaceae, Sapotaceae, and Icacinaceae; Old World Tropic distribution, such as Pittosporaceae, Pandanaceae, and Musaceae; tropical Asian and tropical American disjunct distribution, including Araliaceae, Elaeocarpaceae, Gesneriaceae, Lardizabalaceae, Staphyleaceae, and Styracaceae; and the tropical Asian distribution, such as Crypteroniaceae, Rafflesiaceae, and Sabiaceae. Families of mainly temperate distribution contribute 17.04% of the total flora, including the ones of north temperate distribution such as Caprifoliaceae, Pinaceae, Aceraceae, Betulaceae, Buxaceae, Cornaceae, Fagaceae, Hamamelidaceae, Juglandaceae, and Salicaceae; East Asia and North America disjunct distribution, such as Magnoliaceae, Nyssaceae, and Saururaceae; and East Asia distribution, such as Actinidiaceae, Cephalotaxaceae, and Stachyuraceae.

Geographic elements at the generic level

Patterns of seed plant distribution of the flora at the generic level are enumerated in Table 3. The genera of tropical Asian distribution, such as *Alphonsea*, *Amoora* Roxb., *Pterospermum*, *Mitrephora*, *Mycetia* Reinw., *Aganosma* (Blume) G. Don, *Chukrasia* A. Juss., *Crypteronia* Blume, and *Knema* Lour., show the highest percentage among all distribution types, contributing to 30.19% of the flora. Genera of pantropic distribution, such as *Gnetum* L., *Beilschmiedia* Nees, *Cryptocarya* R. Br., *Capparis* L., *Piper*, *Croton* L., *Dioscorea*, *Uncaria* Schreb., *Lasianthus*, *Morinda* L., *Ardisia*, *Bauhinia* L., and *Marsdenia* R. Br., contribute to 21.34% of the flora.

Table 4. Similarity coefficients at family, generic, and species levels for three tropical regions of Yunnan.[1, 2]

Compared regional flora	Southern Yunnan[3] Shared/similarity coefficient, %	Southwestern Yunnan[4] Shared/similarity coefficient, %	Tropical southeastern Yunnan[5] Shared/similarity coefficient, %
Similarity coefficients at family level			
Southern Yunnan	100/100		
Southwestern Yunnan	180/98.4	100/100	
Tropical southeastern Yunnan	163/89.1	179/96.2	100/100
Similarity coefficients at generic level			
Southern Yunnan	100/100		
Southwestern Yunnan	946/80.4	100/100	
Tropical southeastern Yunnan	939/79.9	1037/76.6	100/100
Similarity coefficients at specific level			
Southern Yunnan	100/100		
Southwestern Yunnan	1834/47.9	100/100	
Tropical southeastern Yunnan	1773/50.7	2165/43.2	100/100

[1] Data are from Zhu et al. (2004) for southwestern Yunnan and Shui (2003) for tropical southeastern Yunnan. There were 4933 species of native seed plants in 1432 and 199 families recognized from southwestern Yunnan (Dehong), and 4996 species of native seed plants in 1355 and 186 families recognized from tropical southeastern Yunnan based on Shui's list.

[2] The similarity coefficient between two regions equals the number of taxa shared by both regions divided by the lowest number of taxa of region 1 or 2, multiplied by 100%.

[3] Southern Yunnan is defined as Xishuangbanna administrative region (site 1 in Fig. 1).

[4] Southwestern Yunnan is defined as Dehong administrative region (site 2 in Fig. 1).

[5] Tropical southeastern Yunnan is defined as the tropical area of southeastern part of Yunnan (site 3 in Fig. 1).

Next are the genera of Old World tropical distribution, such as *Thunbergia* Retz., *Dracaena* Vand. ex L., *Pandanus* Parkinson, *Ventilago* Gaertn., *Stephania* Lour., *Fissistigma*, *Polyalthia* Blume, *Barringtonia* J. R. Forst. & G. Forst., *Carallia* Roxb., *Canarium* L., *Chasalia* DC., and *Uvaria* L. Genera with distributions from tropical Asia to tropical Australia include *Ailanthus* Desf., *Hoya* R. Br., *Argyreia* Lour., *Dillenia* L., *Lagerstroemia* L., *Loeseneriella* A. C. Sm., *Murraya* J. König ex L., and *Toona* (Endl.) M. Roem. Genera with tropical Asian to tropical African distribution include *Bombax* L., *Flacourtia* Comm. ex L'Hér., *Quisqualis* L., *Bridelia* Willd., *Premna* L., *Urophyllum* Jack ex Wall., *Strophanthus* DC., *Mitragyna* Korth., *Garcinia*, *Anogeissus* (DC.) Wall. ex Guill., Perr. & A. Rich., and *Cymbopogon* Spreng. Genera of tropical distribution in all (Table 3, types 2–7) comprise 78.2% of the total number, while genera of temperate distribution in all (Table 3, types 8–14) contribute 15.82% of the total genera, including genera with north temperate distribution, such as *Artemisia* L., *Carpinus* L., *Betula* L., *Salix* L., *Swida* Opiz, *Corydalis* DC., *Pinus* L., and *Sorbus* L., those with a disjunct distribution in East Asia and North America, such as *Schisandra* Michx., *Photinia* Lindl., *Nyssa* L., *Osmanthus* Lour., *Magnolia* L., *Mahonia* Nutt., *Illicium* L., and *Castanopsis*, those of Old World temperate distribution such as *Ajuga* L., *Elsholtzia* Moench, *Herminium* L., *Inula* L., *Ligustrum* L., and *Paris* L., and East Asian distributions such as *Actinidia* Lindl., *Belamcanda* Adans., *Aspidistra* Ker Gawl., *Cephalotaxus* Siebold & Zucc. ex Endl., *Choerospondias* B. L. Burtt & A. W. Hill, *Gardneria* Wall., *Hovenia* Thunb., *Pegia* Colebr., *Skimmia* Thunb., *Stachyurus* Siebold & Zucc., and *Pterocarya* Kunth. Only 11 genera are endemic to China, including *Biondia* Schltr., *Camptotheca* Decne., *Craspedolobium* Harms, *Cyphotheca* Diels, *Dichotomanthes* Kurz, *Eleutharrhena* Forman, *Nouelia* Franch., *Paramomum* S. Q. Tong, *Styrophyton* S. Y. Hu, *Tapiscia* Oliv., and *Thyrocarpus* Hance.

Comparison with Tropical Floras of Southwestern and Southeastern Yunnan

The tropical area in southwestern Yunnan is located mainly in the Dehong administrative region (Fig. 1, site 2), which borders Burma and lies between 23°50′–25°20′N and 97°31′–98°43′E. The region is 11,229 km^2 in area and has a monsoon climate with an annual mean temperature of 19.2°C and an annual precipitation of 1540 mm. There have been 4933 species of 1432 genera and 199 families of native seed plants recognized from the region (Zhu et al., 2004).

The tropical area in southeastern Yunnan referred to in this study (Fig. 1, site 3) is located between the Tropic of Cancer and the Yunnan–Vietnam border, 22°26′–23°26′ N and 104°27′–108°48′E. The region, including six counties, such as Pingbian, Hekou, Jingping, Luchun, Yuanyang, and Honghe, is 14,389 km^2 in area. It also has a monsoon climate with an annual mean temperature of 22.8°C and an annual precipitation of 1764 mm. From Shui (2003),

Table 5. Top 20 families with the highest species richness among the tropical floras of southern, southwestern, and southeastern Yunnan.[1, 2]

Flora of southern Yunnan[3]		Flora of southwestern Yunnan[4]		Flora of tropical southeastern Yunnan[5]	
Family	No. of species (%)[6]	Family	No. of species (%)[6]	Family	No. of species (%)[6]
Orchidaceae	328 (9.82)	Fabaceae	297 (6.02)	Fabaceae	285 (5.70)
Fabaceae	211 (6.32)	Poaceae	269 (5.45)	Orchidaceae	276 (5.52)
Rubiaceae	142 (4.25)	Asteraceae	267 (5.41)	Rubiaceae	232 (4.64)
Poaceae	132 (3.95)	Orchidaceae	259 (5.25)	Poaceae	219 (4.38)
Euphorbiaceae	117 (3.50)	Rubiaceae	166 (3.36)	Asteraceae	196 (3.92)
Asteraceae	106 (3.17)	Rosaceae	153 (3.10)	Lauraceae	141 (2.82)
Moraceae	73 (2.19)	Euphorbiaceae	110 (2.23)	Urticaceae	134 (2.68)
Lauraceae	70 (2.10)	Lauraceae	101 (2.05)	Euphorbiaceae	126 (2.52)
Urticaceae	72 (2.16)	Cyperaceae	97 (1.96)	Rosaceae	124 (2.48)
Zingiberaceae	72 (2.16)	Urticaceae	90 (1.82)	Fagaceae	109 (2.18)
Acanthaceae	68 (2.04)	Moraceae	89 (1.80)	Moraceae	104 (2.08)
Lamiaceae	61 (1.83)	**Ericaceae**	85 (1.72)	**Ericaceae**	96 (1.92)
Asclepiadaceae	58 (1.74)	Acanthaceae	77 (1.56)	Lamiaceae	91 (1.82)
Apocynaceae	51 (1.53)	**Theaceae**	71 (1.44)	Cyperaceae	89 (1.78)
Annonaceae	49 (1.47)	**Liliaceae**	71 (1.44)	**Theaceae**	81 (1.62)
Verbenaceae	48 (1.44)	**Scrophulariaceae**	69 (1.40)	**Gesneriaceae**	79 (1.58)
Cucurbitaceae	46 (1.38)	Fagaceae	68 (1.38)	Acanthaceae	77 (1.54)
Rosaceae	46 (1.38)	**Araliaceae**	66 (1.34)	**Celastraceae**	65 (1.30)
Vitaceae	45 (1.35)	**Apiaceae**	66 (1.34)	**Araliaceae**	64 (1.28)
Fagaceae	44 (1.32)	Asclepiadaceae	64 (1.30)	Verbenaceae	62 (1.24)
Sum of the top 20 families	1839 (55.10)	2535 (51.38)		2650 (53.04)	
All other families	1501 (44.90)	2398 (48.62)		2346 (46.6)	
Total flora	3340 (100)	4933 (100)		4996 (100)	

[1] Data are from Zhu et al. (2004) for southwestern Yunnan and Shui (2003) for the tropical southeastern Yunnan.
[2] Families in boldface are the top 20 families, which can be found only in the flora of Xishuangbanna, in both the floras of Dehong and tropical southeastern Yunnan, or in one of them, respectively.
[3] Southern Yunnan is defined as Xishuangbanna administrative region (site 1 in Fig. 1).
[4] Southwestern Yunnan is defined as Dehong administrative region (site 2 in Fig. 1).
[5] Tropical Southeastern Yunnan is defined as the tropical area of southeastern part of Yunnan (site 3 in Fig. 1).
[6] Number of species for each family in the respective floras, and their percentage within the respective floras.

we compiled a list of 4996 species of 1355 genera and 186 families of native seed plants from the region in southeastern Yunnan.

Floristic similarities at the family, generic, and species levels between the floras of southern Yunnan (Xishuangbanna), southwestern Yunnan (Dehong), and southeastern Yunnan (Fig. 1, sites 1, 2, and 3, respectively) are detailed in Table 4. The floristic similarity between these regional floras of tropical Yunnan is more than 89% at the family level and more than 76% at the generic level, but it is lower at the species level (43%–50%).

The comparison of the 20 families with the most species among these tropical floras of southern, southwestern, and southeastern Yunnan is enumerated in Table 5.

The top-ranking families in all three regional floras are basically similar. These three regional floras belong to the same floristic unit and represent the tropical flora of Yunnan. The families Zingiberaceae, Apocynaceae, Annonaceae, and Cucurbitaceae are in the top 20 ranking families of the flora of southern Yunnan (Fig. 1, site 1), while Ericaceae, Theaceae, and Araliaceae are in the top 20 ranking families of these floras of southwestern and southeastern Yunnan (Fig. 1, sites 2 and 3, respectively).

Comparison of geographic elements at the generic level from these regional floras revealed that the tropical elements (Table 6, types 2–7) contribute more than 64% of the total genera in these regional floras of Yunnan, and the highest proportion of the tropical elements occurs in the flora of southern Yunnan (making up 78.3% of all the genera).

Comparison with Tropical Floras of Mainland Southeast Asia and Malesia

A Catalogue of the Vascular Plants of Malaya (Turner, 1995) and *Thai Plant Names* (Smitinand, 2001) provide recently updated and relatively com-

Table 6. Comparison of geographic elements at the generic level from the tropical floras of southern, southwestern, and southeastern Yunnan.

Geographical elements of genera	Southern Yunnan[1] No. of genera (%)[4]	Southwestern Yunnan[2] No. of genera (%)[4]	Tropical southeastern Yunnan[3] No. of genera (%)[4]
1. Cosmopolitan	59 (5.0)	76 (5.3)	62 (4.6)
2. Pantropic	251 (21.3)	267 (18.6)	239 (17.6)
3. Tropical Asia and tropical America disjunctions	30 (2.6)	30 (2.1)	29 (2.1)
4. Old World Tropics	112 (9.5)	120 (8.4)	116 (8.6)
5. Tropical Asia to tropical Australia	76 (6.5)	74 (5.2)	82 (6.1)
6. Tropical Asia to tropical Africa	96 (8.2)	94 (6.6)	95 (7.0)
7. Tropical Asia	355 (30.2)	340 (23.7)	371 (27.4)
8. North temperate	60 (5.1)	157 (11.0)	111 (8.2)
9. East Asia and North America disjunctions	32 (2.7)	54 (3.8)	47 (3.5)
10. Old World temperate	24 (2.0)	50 (3.5)	32 (2.4)
11. Temperate Asia	5 (0.4)	11 (0.8)	6 (0.4)
12. Mediterranean region, western to central Asia	2 (0.2)	5 (0.3)	3 (0.2)
13. Central Asia	1 (0.1)	4 (0.3)	1 (0.1)
14. East Asia	62 (5.3)	125 (8.7)	120 (8.9)
15. Endemic to China	11 (0.9)	25 (1.7)	41 (3.0)
Total	1176 (100)	1432 (100)	1355 (100)

[1] Southern Yunnan is defined as Xishuangbanna administrative region (site 1 in Fig. 1).
[2] Southwestern Yunnan is defined as Dehong administrative region (site 2 in Fig. 1).
[3] Tropical southeastern Yunnan is defined as the tropical area of the southeastern part of Yunnan (site 3 in Fig. 1).
[4] Number of genera for each geographical element in the respective floras, and their percentage within the respective floras.

plete data on the regional flora of mainland Southeast Asia and Malaysia, respectively. Comparisons of the floristic composition and similarities at the family and generic levels between southern Yunnan (Xishuangbanna), Thailand, and the Malay Peninsula are compared in Tables 7 and 8.

Most of the top 20 families with the most species richness in the flora of southern Yunnan (Xishuangbanna) are shared by the floras of Thailand and the Malay Peninsula as the top 20 families (Table 7). The families Orchidaceae, Rubiaceae, Euphorbiaceae, Lauraceae, Moraceae, Apocynaceae, Annonaceae, Zingiberaceae, and Acanthaceae are large tropical families shared by both southern Yunnan (Xishuangbanna) and Thailand–Malaysia. However, those families with strongly tropical characteristics such as Dipterocarpaceae, Melastomataceae, Myrtaceae, Arecaceae, and Clusiaceae are also dominant in the Thai–Malaysian flora, while Urticaceae, Lamiaceae, Cucurbitaceae, Rosaceae, and Vitaceae, which are of tropical to temperate distributions, are dominant in southern Yunnan (Xishuangbanna). The families with strongly tropical characteristics have a small number of species in the flora of southern Yunnan, but are highly abundant there (Zhu, 1997). The floristic similarity between the flora of southern Yunnan and the floras of Thailand and Malaysia exceeds 82.5% at the family level and 65.6% at the generic level (Table 8).

Discussion

Tropical genera comprise a majority (78.2%) of the southern Yunnan (Xishuangbanna) flora. The dominant genera are those of tropical Asian distribution (Table 3). This reveals that the flora of southern Yunnan is tropical in nature and has strong tropical Asian affinity.

The tropical floras from southwestern, southern, and southeastern Yunnan (Fig. 1, sites 1–3) are similar in families with the most species richness and have high similarities at family and generic levels (more than 89% at the family level and more than 76% at the generic level; see Table 4). The tropical elements comprise more than 64% of the total genera, of which the tropical Asian element contributes more than 23.7% of the total genera in these regional floras of tropical Yunnan. These patterns suggest that these three regions of Yunnan belong to the same floristic unit and are part of tropical Asian or Indo-Malaysian flora.

In comparing the flora of southern Yunnan (Xishuangbanna) to the tropical floras of Guangxi in southern China and Hainan in southeastern China (Zhu & Roos, 2004), the top-ranking families are similar, except that the family Cucurbitaceae is in the top 20 ranking families of the flora of southern Yunnan only, and Theaceae, Myrsinaceae, Liliaceae, and Myrtaceae (in Hainan) are in the top 20 families of those of southern and southeastern China. When

Table 7. Top 20 families with the most species richness among the tropical floras of southern Yunnan, Thailand, and the Malay Peninsula.[1, 2]

Flora of southern Yunnan (Xishuangbanna)		Flora of Thailand		Flora of the Malay Peninsula	
Family	No. of species (%)[3]	Family	No. of species (%)[3]	Family	No. of species (%)[3]
Orchidaceae	328 (9.82)	Orchidaceae	530 (8.67)	Orchidaceae	853 (11.16)
Fabaceae	211 (6.32)	Fabaceae	398 (6.51)	Rubiaceae	562 (7.35)
Rubiaceae	142 (4.25)	Euphorbiaceae	365 (5.97)	Euphorbiaceae	369 (4.81)
Poaceae	132 (3.95)	Rubiaceae	357 (5.84)	Fabaceae	298 (3.90)
Euphorbiaceae	117 (3.50)	Poaceae	227 (3.71)	Poaceae	238 (3.11)
Asteraceae	106 (3.17)	**Cyperaceae**	222 (3.63)	**Myrtaceae**	215 (2.81)
Moraceae	73 (2.19)	**Arecaceae**	167 (2.73)	Lauraceae	214 (2.80)
Lauraceae	70 (2.10)	**Araceae**	151 (2.47)	Annonaceae	202 (2.64)
Urticaceae	72 (2.16)	Apocynaceae	120 (1.96)	**Arecaceae**	198 (2.59)
Zingiberaceae	72 (2.16)	Annonaceae	117 (1.91)	**Gesneriaceae**	189 (2.47)
Acanthaceae	68 (2.04)	Zingiberaceae	112 (1.83)	**Melastomataceae**	172 (2.25)
Lamiaceae	61 (1.83)	**Scrophulariaceae**	103 (1.69)	**Cyperaceae**	162 (2.12)
Asclepiadaceae	58 (1.74)	Moraceae	103 (1.69)	Acanthaceae	158 (2.07)
Apocynaceae	51 (1.53)	Asteraceae	100 (1.64)	**Dipterocarpaceae**	156 (2.04)
Annonaceae	49 (1.47)	Fagaceae	100 (1.64)	Zingiberaceae	153 (2.00)
Verbenaceae	48 (1.44)	**Myrtaceae**	96 (1.57)	**Araceae**	141 (1.84)
Cucurbitaceae	46 (1.38)	Verbenaceae	83 (1.36)	Moraceae	138 (1.81)
Rosaceae	46 (1.38)	Lauraceae	82 (1.34)	**Clusiaceae**	120 (1.57)
Vitaceae	45 (1.35)	Acanthaceae	80 (1.31)	Apocynaceae	119 (1.56)
Fagaceae	44 (1.32)	**Convolvulaceae**	78 (1.28)	Asclepiadaceae	116 (1.52)
Sum of the top 20 families	1839 (55.10)		3591 (58.75)		4773 (62.42)
All other families	1501 (44.90)		2520 (41.25)		2864 (37.58)
Total flora	3340 (100)		6111 (100)		7637 (100)

[1] Data are from Smitinand (2001) for Thailand and from Turner (1995) for the Malay Peninsula. There were 6111 species of native seed plants in 1573 genera and 198 families recognized from Smitinand's list, and 7637 species of native seed plants in 1554 genera and 191 families recognized from Turner's list. Circumscription of families and nomenclature follow the Missouri Botanical Garden's w3TROPICOS (<http://www.tropicos.org>).

[2] Families in boldface are the top 20 families, which can be found only in the flora of Xishuangbanna, in both the floras of Thailand and the Malay Peninsula, or in one of them, respectively.

[3] Number of species for each family in the respective floras, and their percentage within the respective floras.

Table 8. Comparison of floristic similarities at the family and generic levels between southern Yunnan, Thailand, and the Malay Peninsula.[1, 2]

	Southern Yunnan	Thailand	Malay Peninsula
Compared flora	Shared/similarity coefficient, %	Shared/similarity coefficient, %	Shared/similarity coefficient, %
Similarity coefficients at family level			
Southern Yunnan (Xishuangbanna)	100/100		
Thailand	174/95.08	100/100	
Malay Peninsula	151/82.51	173/90.58	100/100
Similarity coefficients at generic level			
Southern Yunnan (Xishuangbanna)	100/100		
Thailand	867/73.72	100/100	
Malay Peninsula	772/65.65	1099/70.72	100/100

[1] Data are from Smitinand (2001) for Thailand and from Turner (1995) for the Malay Peninsula.

[2] The similarity coefficient between two regions equals the number of taxa shared by both regions divided by the lowest number of taxa of region 1 or 2, multiplied by 100%.

Table 9. Comparison of the distribution types of genera from the flora of southern Yunnan (Xishuangbanna), the flora of tropical Guangxi, and the flora of Hainan.[1]

	Southern Yunnan (Xishuangbanna)	Tropical Guangxi[2]	Hainan
Distribution types (geographic elements)	Genera %	Genera %	Genera %
1. Cosmopolitan	5.02	5.56	5.09
2. Pantropic	21.34	19.71	23.42
3. Tropical Asia and tropical America disjunctions	2.55	2.24	2.75
4. Old World Tropics	9.52	9.35	11.39
5. Tropical Asia to tropical Australia	6.46	7.42	10.26
6. Tropical Asia to tropical Africa	8.16	7.19	7.03
7. Tropical Asia	30.19	25.81	25.93
8. North temperate	5.1	6.8	4.44
9. East Asia and North America disjunctions	2.72	3.48	2.99
10. Old World temperate	2.04	2.55	1.94
11. Temperate Asia	0.43	0.46	0.32
12. Mediterranean region, western to central Asia	0.17	0.15	0.16
13. Central Asia	0.09	0.00	0.00
14. East Asia	5.27	6.65	2.75
15. Endemic to China	0.94	2.63	1.53
Total	100	100	100.00

[1] Data are from Fang et al. (1995) for tropical Guangxi (1294 genera) and from Wu et al. (1996) for Hainan (1238 genera).
[2] Tropical Guangxi is in the southwestern part of Guangxi province in southern China.

comparing geographic elements at the generic level, the tropical elements contribute to more than 71% of the total genera in all these tropical floras of China (Table 9).

Most of the dominant families from the flora of southern Yunnan (Xishuangbanna) are also dominant in mainland Southeast Asian and Malaysian floras. The similarities between the flora of southern Yunnan and these floras of tropical Asia are more than 82.5% at the family level and more than 65.6% at the generic level (Table 8). This pattern indicates that the tropical flora of southern Yunnan is similar to the Indo-Malaysian flora and belongs to the Indo-Malaysian or Malesian floristic region as suggested by Takhtajan (1988) and Wu and Wu (1996). In our study, these floras of southern Yunnan, Thailand, and the Malay Peninsula comprise a floristic continuum. We agree with van Balgooy that the number of taxa in common is the first step in surveying floristic affinity (van Balgooy et al., 1996). The higher percentage of taxa shared by these floras suggests their close floristic affinity.

The flora of southern Yunnan occurs on the margin of tropical Asia. However, although tropical families and genera contribute most to its total flora, the taxa of strictly tropical distribution are still under-represented compared to the Malaysian flora. For example, Dipterocarpaceae has only two species from two genera, respectively, in the flora of southern Yunnan, although these taxa are the most abundant trees in some forest types (Zhu, 1992).

The flora of southern Yunnan is similar to both the Tertiary flora and the present flora of northeast India. The Tertiary flora of northeast India, such as *Syzygium*, *Terminalia*, *Artocarpus* J. R. Forst. & G. Forst., *Ficus*, *Mangifera* L., *Bombax*, *Garcinia*, *Calophyllum* L., *Nephelium* L., *Rhizophora* L., *Memecylon* L., *Barringtonia*, *Canarium*, *Pometia*, *Sterculia* L., and *Garuga* Roxb. (Mehrotra et al., 2005), which are frequent genera in the flora of southern Yunnan, were formed by tropical elements. A floristic comparison between the dipterocarp forest in southern Yunnan (Xishuangbanna) and the dipterocarp forest in the upper Assam (Rajkhowa, 1961) revealed that the floristic similarities are 97.3% at the family level and 79.7% at the generic level (Zhu, 1994). The vegetation history of southern Yunnan (Xishuangbanna) during the Tertiary could not be accurately portrayed because of the lack of paleobotanical data. However, the available information from the neighboring regions suggested that southern Yunnan could be a subtropical evergreen broad-leaved forest during the Tertiary. A 40,000-year palynological record from northeast Thailand indicated that the region supported a Fagaceae-coniferous forest, similar to contemporary vegetation from subtropical southwest China, and climatic conditions were cooler and probably drier in the Pleistocene than in present-day northern Thailand (Penny, 2001). Southern Yunnan is geographically near northern Thailand, so it is possible that southern Yunnan had similar vegetation and climatic condi-

tions during the Pleistocene. The migration of tropical Asian elements into southern Yunnan could have mainly taken place after the Tertiary. The tropical flora of southern Yunnan is believed to be derived from tropical Asian flora with the uplift of the Himalayas and the formation of the eastern monsoon climate after the Tertiary.

Studies on the geological history of Southeast Asia reveal that the direct land connection between mainland Southeast Asia and western Malesia existed until the early Pliocene 5 million years ago (Hall, 1998), and there was no geographical barrier between southern Yunnan, mainland Southeast Asia, and western Malesia during most of the Tertiary (Morley, 1998). Thus, geology contributes to and underlies the close affinity between the flora of southern Yunnan and the flora of Malaysia.

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Appendix 1. Genera list of the flora of southern Yunnan (Xishuangbanna). Numbers in parentheses after the family and genus names indicate the inclusive number of genera and species, respectively.

Families	Genera
Acanthaceae (32)	*Acanthus* L. (1), *Adhatoda* Mill. (1), *Andrographis* Wall. ex Nees (2), *Asystasia* Blume (2), *Asystasiella* Lindau (1), *Baphicacanthus* Bremek. (1), *Barleria* L. (1), *Calophanoides* Ridl. (1), *Chroesthes* Benoist (2), *Codonacanthus* Nees (2), *Dicliptera* Juss. (2), *Eranthemum* L. (1), *Goldfussia* Nees (1), *Hemigraphis* Nees (1), *Hygrophila* R. Br. (1), *Hypoestes* Sol. ex R. Br. (1), *Isoglossa* Oerst. (1), *Lepidagathis* Willd. (1), *Mananthes* Bremek. (1), *Nelsonia* R. Br. (1), *Ophiorrhiziphyllon* Kurz (1), *Peristrophe* Nees (1), *Phaulopsis* Willd. (1), *Phlogacanthus* Nees (2), *Pseuderanthemum* Radlk. (3), *Pteracanthus* (Nees) Bremek. (1), *Rhaphidosperma* G. Don (1), *Rostellularia* Rchb. (2), *Rungia* Nees (4), *Sericocalyx* Bremek. (1), *Strobilanthes* Blume (21), *Thunbergia* Retz. (5)
Aceraceae (1)	*Acer* L. (4).
Actinidiaceae (2)	*Actinidia* Lindl. (2), *Saurauia* Willd. (6)
Aizoaceae (2)	*Glinus* L. (2), *Mollugo* L. (1)
Alismataceae (2)	*Caldesia* Parl. (1), *Sagittaria* L. (2)
Amaranthaceae (7)	*Achyranthes* L. (4), *Aerva* Forssk. (1), *Alternanthera* Forssk. (2), *Amaranthus* L. (3), *Celosia* L. (1), *Cyathula* Blume (1), *Deeringia* R. Br. (1)
Amaryllidaceae (2)	*Curculigo* Gaertn. (2), *Hypoxis* L. (1)
Anacardiaceae (12)	*Buchanania* Spreng. (1), *Choerospondias* B. L. Burtt & A. W. Hill (1), *Dracontomelon* Blume (1), *Drimycarpus* Hook. f. (1), *Lannea* A. Rich. (1), *Mangifera* L. (2), *Pegia* Colebr. (1), *Pistacia* L. (1), *Rhus* L. (2), *Semecarpus* L. f. (2), *Spondias* L. (2), *Toxicodendron* Mill. (3)

Appendix 1. Continued.

Families	Genera
Annonaceae (15)	*Alphonsea* Hook. f. & Thomson (5), *Artabotrys* R. Br. ex Ker Gawl. (2), *Cyathostemma* Griff. (1), *Dasymaschalon* Dalla Torre & Harms (1), *Desmos* Lour. (3), *Fissistigma* Griff. (9), *Goniothalamus* (Blume) Hook. f. & Thomson (2), *Mezzettiopsis* Ridl. (1), *Miliusa* Lesch. (6), *Mitrephora* Hook. f. & Thomson (3), *Orophea* Blume (1), *Phaeanthus* Hook. f. & Thomson (1), *Polyalthia* Blume (8), *Pseuduvaria* Miq. (1), *Uvaria* L. (5)
Apiaceae (8)	*Centella* L. (1), *Eryngium* L. (1), *Heracleum* L. (1), *Hydrocotyle* L. (6), *Oenanthe* L. (3), *Pimpinella* L. (4), *Sanicula* L. (1), *Trachyspermum* Link (1)
Apocynaceae (23)	*Alganosma* (Blume) G. Don (4), *Alstonia* R. Br. (2), *Alyxia* Banks ex R. Br. (4), *Amalocalyx* Pierre (1), *Anodendron* A. DC. (2), *Beaumontia* Wall. (2), *Bousigonia* Pierre (2), *Chonemorpha* G. Don (1), *Cleghornia* Wight (1), *Epigynum* Wight (1), *Holarrhena* R. Br. (1), *Ichnocarpus* R. Br. (2), *Kibatalia* G. Don (2), *Kopsia* Blume (1), *Melodinus* J. R. Forst. & G. Forst. (2), *Parameria* Benth. (1), *Pottsia* Hook. & Arn. (1), *Rauvolfia* L. (1), *Strophanthus* DC. (2), *Tabernaemontana* L. (3), *Trachelospermum* Lem. (3), *Urceola* Vand. (4), *Wrightia* R. Br. (5)
Aquifoliaceae (1)	*Ilex* L. (12)
Araceae (17)	*Acorus* L. (2), *Aglaonema* Schott (1), *Alocasia* (Schott) G. Don (2), *Amorphophallus* Blume ex Decne. (7), *Arisaema* Mart. (4), *Colocasia* Schott (1), *Cryptocoryne* Fischer ex Wydler (1), *Epipremnum* Schott (1), *Gonatanthus* Klotzsch (1), *Homalomena* Schott (2), *Lasia* Lour. (1), *Pistia* L. (1), *Pothos* L. (3), *Remusatia* Schott (2), *Rhaphidophora* Hassk. (8), *Steudnera* K. Koch (1), *Typhonium* Schott (2)
Araliaceae (10)	*Aralia* L. (5), *Brassaiopsis* Decne. & Planch. (3), *Eleutherococcus* Maxim. (1), *Euaraliopsis* Hutch. (2), *Heteropanax* Seem. (2), *Macropanax* Miq. (3), *Panax* L. (2), *Schefflera* J. R. Forst. & G. Forst. (6), *Trevesia* Vis. (1), *Tupidanthus* Hook. f. & Thomson (1)
Arecaceae (8)	*Arenga* Labill. (1), *Calamus* L. (24), *Caryota* L. (3), *Livistona* R. Br. (1), *Phoenix* L. (2), *Pinanga* Blume (4), *Plectocomia* Mart. ex Blume (3), *Wallichia* Roxb. (2)
Aristolochiaceae (1)	*Aristolochia* L. (6)
Asclepiadaceae (26)	*Asclepias* L. (1), *Biondia* Schltr. (1), *Brachystelma* R. Br. (1), *Calotropis* R. Br. (1), *Ceropegia* L. (2), *Cryptolepis* R. Br. (2), *Cynanchum* L. (3), *Dischidia* R. Br. (2), *Dregea* E. Mey. (1), *Genianthus* Hook. f. (1), *Gongronema* (Endl.) Decne. (1), *Goniostemma* Wight (1), *Gymnema* R. Br. (4), *Heterostemma* Wight & Arn. (7), *Hoya* R. Br. (8), *Marsdenia* R. Br. (7), *Metaplexis* R. Br. (1), *Micholitzia* N. E. Br. (1), *Myriopteron* Griff. (1), *Oxystelma* R. Br. (1), *Pentasachme* Wall. ex Wight (1), *Raphistemma* Wall. (1), *Stelmocrypton* Baill. (1), *Streptocaulon* Wight & Arn. (1), *Toxocarpus* Wight & Arn. (4), *Tylophora* R. Br. (3)
Asteraceae (59)	*Acanthospermum* Schrank (2), *Achillea* L. (1), *Adenostemma* J. R. Forst. & G. Forst. (2), *Ageratum* L. (1), *Arctium* L. (1), *Artemisia* L. (4), *Bidens* L. (4), *Blainvillea* Cass. (1), *Blumea* DC. (9), *Blumeopsis* Gagnep. (1), *Camchaya* Gagnep. (1), *Carpesium* L. (1), *Centipeda* Lour. (1), *Chromolaena* DC. (1), *Cirsium* Mill. (1), *Conyza* Less. (4), *Cotula* L. (1), *Crassocephalum* Moench (1), *Crepis* L. (1), *Cyathocline* Cass. (1), *Dichrocephala* L'Hér. ex DC. (3), *Eclipta* L. (1), *Elephantopus* L. (1), *Emilia* Cass. (1), *Enydra* Lour. (1), *Ethulia* L. f. (1), *Eupatorium* L. (1), *Galinsoga* Ruiz & Pav. (1), *Gerbera* L. (1), *Gnaphalium* L. (3), *Gochnatia* Kunth (1), *Grangea* Adans. (1), *Gynura* Cass. (2), *Hemisteptia* Bunge ex Fisch. & C. A. Mey. (1), *Inula* L. (1), *Ixeridium* (A. Gray) Tzvelev (1), *Lactuca* L. (1), *Laggera* Sch. Bip. ex Benth. & Hook. f. (2), *Microglossa* DC. (1), *Nouelia* Franch. (1), *Pentanema* Cass. (2), *Picris* L. (1), *Pluchea* Cass. (1), *Pterocypsela* C. Shih (1), *Senecio* L. (1), *Siegesbeckia* Steud. (3), *Sonchus* L. (1), *Sphaeranthus* L. (3), *Sphaeromorphaea* DC. (1), *Spilanthes* Jacq. (2), *Synedrella* Gaertn. (1), *Synotis* (C. B. Clarke) C. Jeffrey & Y. L. Chen (1), *Thespis* DC. (1), *Tricholepis* DC. (1), *Tridax* L. (1), *Vernonia* Schreb. (14), *Wedelia* Jacq. (2), *Xanthium* L. (1), *Youngia* Cass. (3)
Balanophoraceae (1)	*Balanophora* J. R. Forst. & G. Forst. (4)
Balsaminaceae (1)	*Impatiens* L. (7)
Begoniaceae (1)	*Begonia* L. (11)
Berberidaceae (1)	*Mahonia* Nutt. (1)
Betulaceae (3)	*Alnus* Mill. (1), *Betula* L. (1), *Carpinus* L. (2)
Bignoniaceae (7)	*Fernandoa* Welw. ex Seem. (1), *Markhamia* Seem. ex Baill. (2), *Mayodendron* Kurz. (1), *Nyctocalos* Teijsm. & Binn. (1), *Oroxylum* Vent. (1), *Radermachera* Zoll. & Moritzi (2), *Stereospermum* Cham. (2)
Bombacaceae (1)	*Bombax* L. (2)
Boraginaceae (9)	*Bothriospermum* Bunge (1), *Cordia* L. (2), *Cynoglossum* L. (2), *Ehretia* P. Browne (5), *Heliotropium* L. (3), *Thyrocarpus* Hance (1), *Tournefortia* L. (2), *Trichodesma* R. Br. (1), *Trigonotis* Steven (1)

Appendix 1. Continued.

Families	Genera
Brassicaceae (3)	*Cardamine* L. (2), *Raphanus* L. (1), *Rorippa* Scop. (2)
Burmanniaceae (1)	*Burmannia* L. (3)
Burseraceae (3)	*Canarium* L. (4), *Garuga* Roxb. (3), *Protium* Burm. f. (1)
Butomaceae (2)	*Butomopsis* Kunth (1), *Limnocharis* Bonpl. (1)
Buxaceae (2)	*Buxus* L. (1), *Sarcococca* Lindl. (1)
Campanulaceae (9)	*Asyneuma* Griseb. & Schenk (1), *Campanula* L. (1), *Campanumoea* Blume (4), *Cephalostigma* A. DC. (1), *Codonopsis* Wall. (1), *Lobelia* L. (6), *Pentaphragma* Wall. ex G. Don (1), *Pratia* Gaudich. (1), *Wahlenbergia* Schrad. ex Roth (1)
Capparaceae (4)	*Borthwickia* W. W. Sm. (1), *Capparis* L. (10), *Crateva* L. (2), *Stixis* Lour. (1)
Caprifoliaceae (5)	*Carlemannia* Benth. (1), *Lonicera* L. (4), *Sambucus* L. (1), *Silvianthus* Hook. f. (1), *Viburnum* L. (7)
Caryophyllaceae (6)	*Brachystemma* D. Don (1), *Drymaria* Willd. ex Schult. (1), *Myosoton* Moench (1), *Polycarpon* Loefl. ex L. (1), *Silene* L. (1), *Stellaria* L. (2)
Celastraceae (4)	*Celastrus* L. (6), *Glyptopetalum* Thwaites (2), *Maytenus* Molina (6), *Microtropis* Wall. ex Meisn. (2)
Cephalotaxaceae (1)	*Cephalotaxus* Siebold & Zucc. ex Endl. (1)
Ceratophyllaceae (1)	*Ceratophyllum* L. (1)
Chenopodiaceae (2)	*Chenopodium* L. (1), *Dysphania* R. Br. (1)
Chloranthaceae (2)	*Chloranthus* Sw. (2), *Sarcandra* Gardner (1)
Clusiaceae (5)	*Calophyllum* L. (1), *Cratoxylum* Blume (2), *Garcinia* L. (6), *Hypericum* L. (3), *Triadenum* Raf. (1)
Combretaceae (4)	*Anogeissus* (DC.) Wall. ex Guill., Perr. & A. Rich. (1), *Combretum* Loefl. (6), *Quisqualis* L. (2), *Terminalia* L. (2)
Commelinaceae (10)	*Amischotolype* Hassk. (2), *Commelina* L. (5), *Cyanotis* D. Don (3), *Dictyospermum* Wight (1), *Floscopa* Lour. (2), *Murdannia* Royle (9), *Pollia* Thunb. (5), *Porandra* D. Y. Hong (2), *Rhopalephora* Hassk. (1), *Streptolirion* Edgew. (1)
Connaraceae (4)	*Connarus* L. (1), *Rourea* Aubl. (1), *Roureopsis* Planch. (1), *Santaloides* G. Schellenb. (1)
Convolvulaceae (13)	*Argyreia* Lour. (12), *Cuscuta* L. (2), *Dichondra* J. R. Forst. & G. Forst. (1), *Dinetus* Buch.-Ham. ex Sweet (1), *Erycibe* Roxb. (2), *Ipomoea* L. (7), *Merremia* Dennst. ex Endl. (5), *Neuropeltis* Wall. (1), *Operculina* Silva Manso (1), *Porana* Burm. f. (1), *Poranopsis* Roberty (1), *Tridynamia* Gagnep. (1), *Xenostegia* D. F. Austin & Staples (1)
Cornaceae (4)	*Alangium* Lam. (6), *Helwingia* Willd. (1), *Mastixia* Blume (3), *Swida* Opiz (1)
Crassulaceae (2)	*Bryophyllum* Salisb. (1), *Kalanchoe* Adans. (1)
Crypteroniaceae (1)	*Crypteronia* Blume (1)
Cucurbitaceae (17)	*Coccinia* Wight & Arn. (1), *Cucumis* L. (2), *Gomphogyne* Griff. (1), *Gymnopetalum* Arn. (2), *Gynostemma* Blume (6), *Hemsleya* Cogn. ex F. B. Forbes & Hemsl. (2), *Hodgsonia* Hook. f. & Thomson (2), *Luffa* Mill. (1), *Momordica* L. (2), *Mukia* Arn. (2), *Neoalsomitra* Hutch. (1), *Siraitia* Merr. (1), *Solena* Lour. (1), *Thladiantha* Bunge (3), *Trichosanthes* L. (14), *Zanonia* L. (1), *Zehneria* Endl. (4)
Cupressaceae (1)	*Calocedrus* Kurz (1)
Cycadaceae (1)	*Cycas* L. (3)
Cyperaceae (14)	*Bulbostylis* Kunth (1), *Carex* L. (2), *Courtoisia* Marchand (1), *Cyperus* L. (14), *Eleocharis* R. Br. (4), *Fimbristylis* Vahl (4), *Fuirena* Rottb. (1), *Kyllinga* Rottb. (2), *Lipocarpha* R. Br. (1), *Mariscus* Vahl (3), *Pycreus* P. Beauv. (2), *Schoenoplectus* (Rchb.) Palla (2), *Scirpus* L. (1), *Scleria* P. J. Bergius (2)
Daphniphyllaceae (1)	*Daphniphyllum* Blume (2)
Datiscaceae (1)	*Tetrameles* R. Br. (1)
Dichapetalaceae (1)	*Dichapetalum* Thouars (1)
Dilleniaceae (2)	*Dillenia* L. (3), *Tetracera* L. (1)
Dioscoreaceae (1)	*Dioscorea* L. (22)
Dipterocarpaceae (2)	*Parashorea* Kurz (1), *Vatica* L. (1)
Droseraceae (1)	*Drosera* L. (1)
Ebenaceae (1)	*Diospyros* L. (6)
Elaeagnaceae (1)	*Elaeagnus* L. (5)
Elaeocarpaceae (2)	*Elaeocarpus* L. (16), *Sloanea* L. (2)
Elatinaceae (2)	*Bergia* L. (1), *Elatine* L. (1)
Ericaceae (4)	*Agapetes* D. Don ex G. Don (4), *Craibiodendron* W. W. Sm. (1), *Rhododendron* L. (4), *Vaccinium* L. (5)
Eriocaulaceae (1)	*Eriocaulon* L. (2)
Erythroxylaceae (1)	*Erythroxylum* P. Browne (1)

Appendix 1. Continued.

Families	Genera
Euphorbiaceae (36)	*Ietephila* Blume (2), *Alchornea* Sw. (2), *Antidesma* L. (10), *Aporosa* Blume (3), *Baccaurea* Lour. (1), *Baliospermum* Blume (2), *Bischofia* Blume (1), *Blachia* Baill. (1), *Breynia* J. R. Forst. & G. Forst. (5), *Bridelia* Willd. (4), *Chaetocarpus* Thwaites (1), *Claoxylon* A. Juss. (4), *Cleidion* Blume (3), *Cleistanthus* Hook. f. ex Planch. (1), *Croton* L. (7), *Dalechampia* L. (1), *Drypetes* Vahl (4), *Epiprinus* Griff. (1), *Euphorbia* L. (3), *Flueggea* Willd. (2), *Glochidion* J. R. Forst. & G. Forst. (12), *Gymnanthes* Sw. (1), *Homonoia* Lour. (1), *Lasiococca* Hook. f. (1), *Macaranga* Thouars (4), *Mallotus* Lour. (14), *Megistostigma* Hook. f. (1), *Ostodes* Blume (3), *Phyllanthodendron* Hemsl. (1), *Phyllanthus* L. (8), *Sapium* Jacq. (5), *Sauropus* Blume (2), *Sumbaviopsis* J. J. Sm. (1), *Suregada* Roxb. ex Rottler (1), *Trewia* L. (1), *Trigonostemon* Blume (3)
Fabaceae (65)	*Abrus* Adans. (1), *Acacia* Mill. (6), *Acrocarpus* Wight & Arn. (1), *Adenanthera* L. (1), *Aeschynomene* L. (1), *Albizia* Durazz. (6), *Alysicarpus* Desv. (2), *Antheroporum* Gagnep. (1), *Bauhinia* L. (9), *Caesalpinia* L. (7), *Cajanus* Adans. (4), *Campylotropis* Bunge (5), *Cassia* L. (2), *Catenaria* Benth. in Miquel (1), *Chamaecrista* Moench (1), *Clitoria* L. (1), *Codariocalyx* Hassk. (2), *Craspedolobium* Harms (1), *Crotalaria* L. (15), *Cylindrokelupha* Kosterm. (3), *Dalbergia* L. f. (6), *Dendrolobium* (Wight & Arn.) Benth. (2), *Derris* Lour. (4), *Desmodium* Desv. (9), *Dunbaria* Wight & Arn. (1), *Entada* Adans. (2), *Eriosema* (DC.) Desv. (1), *Erythrina* L. (2), *Euchresta* Benn. (2), *Flemingia* Roxb. ex W. T. Aiton (11), *Fordia* Hemsl. (2), *Gleditsia* L. (1), *Gueldenstaedtia* Fisch. (1), *Indigofera* L. (6), *Lespedeza* Michx. (1), *Mecopus* Benn. (1), *Melilotus* Mill. (1), *Millettia* Wight & Arn. (16), *Mimosa* L. (1), *Mucuna* Adans. (6), *Nicolsonia* DC. (11), *Ormosia* Jacks. (5), *Pachyrhizus* Rich. ex DC. (1), *Phaseolus* L. (1), *Phylacium* Benn. (1), *Phyllodium* Desv. (3), *Pithecolobium* Benth. (1), *Podocarpium* (Benth.) Yen C. Yang & P. H. Huang (3), *Priotropis* Wight & Arn. (1), *Psoralea* L. (1), *Pueraria* DC. (5), *Pycnospora* R. Br. ex Wight & Arn. (1), *Rhynchosia* Lour. (1), *Senna* Mill. (3), *Sesbania* Scop. (2), *Shuteria* Wight & Arn. (3), *Smithia* Aiton (2), *Sophora* L. (5), *Spatholobus* Hassk. (4), *Tadehagi* H. Ohashi (2), *Tephrosia* Pers. (2), *Uraria* Desv. (4), *Urariopsis* Schindl. (1), *Whitfordiodendron* Elmer (1), *Zornia* J. F. Gmel. (1)
Fagaceae (5)	*Castanopsis* (D. Don) Spach (16), *Cyclobalanopsis* Oerst. (6), *Lithocarpus* Blume (16), *Quercus* L. (5), *Trigonobalanus* Forman (1)
Flacourtiaceae (6)	*Bennettiodendron* Merr. (1), *Casearia* Jacq. (5), *Flacourtia* Comm. ex L'Hér. (4), *Homalium* Jacq. (2), *Hydnocarpus* Gaertn. (1), *Xylosma* G. Forst. (3)
Fumariaceae (1)	*Corydalis* DC. (2)
Gentianaceae (5)	*Canscora* Lam. (1), *Exacum* L. (1), *Gentiana* L. (1), *Swertia* L. (1), *Tripterospermum* Blume (1)
Geraniaceae (1)	*Geranium* L. (1)
Gesneriaceae (13)	*Aeschynanthus* Jack (10), *Chirita* Buch.-Ham. ex D. Don (4), *Didissandra* C. B. Clarke (1), *Didymocarpus* Wall. (1), *Epithema* Blume (1), *Leptoboea* C. B. Clarke (1), *Oreocharis* Benth. (1), *Ornithoboea* Parish ex C. B. Clarke (2), *Paraboea* (C. B. Clarke) Ridl. (3), *Petrocosmea* Oliv. (1), *Rhynchoglossum* Blume (1), *Rhynchotechum* Blume (2), *Trisepalum* C. B. Clarke (1)
Gnetaceae (1)	*Gnetum* L. (3)
Grossulariaceae (1)	*Polyosma* Blume (1)
Hamamelidaceae (2)	*Ltingia* Noronha (1), *Distyliopsis* P. K. Endress (1)
Hernandiaceae (1)	*Illigera* Blume (7)
Hippocrateaceae (3)	*Loeseneriella* A. C. Sm. (3), *Pristimera* Miers (3), *Salacia* L. (5)
Hydrangeaceae (1)	*Dichroa* Lour. (1)
Hydrocharitaceae (1)	*Blyxa* Noronha ex Thouars (2), *Hydrilla* Rich. (1), *Hydrocharis* L. (1), *Ottelia* Pers. (1)
Hydrophyllaceae (1)	*Hydrolea* L. (1)
Icacinaceae (8)	*Apodytes* E. Mey. ex Arn. (1), *Gomphandra* Wall. ex Lindl. (1), *Iodes* Blume (2), *Mappianthus* Hand.-Mazz. (1), *Natsiatopsis* Kurz (1), *Nothapodytes* Blume (3), *Peripterygium* Hassk. (2), *Pittosporopsis* Craib (1)
Iridaceae (2)	*Belamcanda* Adans. (1), *Iris* L. (3)
Juglandaceae (3)	*Engelhardia* Lesch. ex Blume (4), *Juglans* L. (2), *Pterocarya* Kunth (1)
Juncaceae (1)	*Juncus* L. (1)
Lamiaceae (28)	*Acrocephalus* Benth. (1), *Ajuga* L. (1), *Anisochilus* Wall. ex Benth. (1), *Anisomeles* R. Br. (1), *Ceratanthus* F. Muell. ex G. Taylor (1), *Clinopodium* L. (1), *Colebrookea* Sm. (1), *Coleus* Lour. (1), *Colquhounia* Wall. (1), *Craniotome* Rchb. (1), *Dysophylla* Blume (1), *Elsholtzia* Willd. (10), *Eurysolen* Prain (1), *Geniosporum* Wall. ex Benth. (1), *Glechoma* L. (1), *Gomphostemma* Benth. (9), *Leonurus* L. (1), *Leucas* R. Br. (3), *Leucosceptrum* Sm. (1), *Microtoena* Prain (2), *Mosla* (Benth.) Buch.-Ham. ex Maxim. (1), *Origanum* L. (1), *Paraphlomis* (Prain) Prain (1), *Pogostemon* Desf. (7), *Rabdosia* Hassk. (6), *Salvia* L. (2), *Scutellaria* L. (2), *Teucrium* L. (1)

Appendix 1. Continued.

Families	Genera
Lardizabalaceae (1)	*Stauntonia* DC. (2)
Lauraceae (14)	*Actinodaphne* Nees (2), *Alseodaphne* Nees (2), *Beilschmiedia* Nees (7), *Camphora* Fabr. (1), *Cassytha* L. (1), *Cinnamomum* Schaeff. (9), *Cryptocarya* R. Br. (5), *Lindera* Thunb. (6), *Litsea* Lam. (21), *Machilus* Nees (1), *Neocinnamomum* H. Liu (1), *Neolitsea* (Benth.) Merr. (1), *Persea* Mill. (5), *Phoebe* Nees (8)
Lecythidaceae (1)	*Barringtonia* J. R. Forst. & G. Forst. (1)
Lemnaceae (3)	*Lemna* L. (2), *Spirodela* Schleid. (1), *Wolffia* Horkel ex Schleid. (1)
Lentibulariaceae (1)	*Utricularia* L. (7)
Liliaceae (19)	*Allium* L. (1), *Asparagus* L. (2), *Aspidistra* Ker Gawl. (1), *Campylandra* Baker (2), *Chlorophytum* Ker Gawl. (2), *Dianella* Lam. ex Juss. (1), *Disporopsis* Hance (1), *Disporum* Salisb. ex D. Don (2), *Dracaena* Vand. ex L. (4), *Gloriosa* L. (1), *Hemerocallis* L. (1), *Heterosmilax* Kunth (1), *Liriope* Lour. (1), *Ophiopogon* Ker Gawl. (7), *Paris* L. (1), *Peliosanthes* Andrews (1), *Polygonatum* Mill. (3), *Reineckea* Kunth (1), *Tupistra* Ker Gawl. (2)
Linaceae (2)	*Ixonanthes* Jack (1), *Reinwardtia* Dumort. (1)
Loganiaceae (6)	*Buddleja* L. (6), *Fagraea* Thunb. (1), *Gardneria* Wall. (2), *Gelsemium* Juss. (1), *Mitreola* L. (1), *Strychnos* L. (4)
Loranthaceae (6)	*Dendrophthoe* Mart. (1), *Elytranthe* (Blume) Blume (1), *Helixanthera* Lour. (4), *Macrosolen* (Blume) Blume (4), *Scurrula* L. (7), *Taxillus* Tiegh. (1)
Lythraceae (4)	*Ammannia* L. (3), *Lagerstroemia* L. (4), *Rotala* L. (2), *Woodfordia* Salisb. (1)
Magnoliaceae (9)	*Alcimandra* Dandy (1), *Illicium* L. (2), *Kadsura* Juss. (1), *Magnolia* L. (1), *Manglietia* Blume (2), *Michelia* L. (2), *Parakmeria* Hu & W. C. Cheng (1), *Paramichelia* Hu (1), *Schisandra* Michx. (3)
Malpighiaceae (2)	*Aspidopterys* A. Juss. ex Endl. (3), *Hiptage* Gaertn. (3)
Malvaceae (10)	*Abelmoschus* Medik. (5), *Abutilon* Mill. (2), *Cenocentrum* Gagnep. (1), *Hibiscus* L. (4), *Kydia* Roxb. (3), *Malva* L. (1), *Malvastrum* A. Gray (1), *Sida* L. (11), *Thespesia* Sol. ex Corrêa (1), *Urena* L. (5)
Marantaceae (3)	*Donax* Lour. (1), *Phrynium* Willd. (2), *Stachyphrynium* K. Schum. (1).
Melastomataceae (9)	*Allomorphia* Blume (2), *Cyphotheca* Diels (1), *Medinilla* Gaudich. ex DC. (4), *Melastoma* L. (3), *Memecylon* L. (3), *Osbeckia* L. (6), *Oxyspora* DC. (2), *Sonerila* Roxb. (5), *Styrophyton* S. Y. Hu (1)
Meliaceae (12)	*Aglaia* Lour. (2), *Amoora* Roxb. (7), *Aphanamixis* Blume (2), *Chisocheton* Blume (1), *Chukrasia* A. Juss. (2), *Cipadessa* Blume (1), *Dysoxylum* Blume (10), *Melia* L. (2), *Munronia* Wight (1), *Toona* (Endl.) M. Roem. (5), *Trichilia* P. Browne (2), *Walsura* Roxb. (2)
Menispermaceae (14)	*Aspidocarya* Hook. f. & Thomson (1), *Cissampelos* L. (1), *Cocculus* DC. (2), *Cyclea* Arn. ex Wight (6), *Diploclisia* Miers (1), *Eleutharrhena* Forman (1), *Hypserpa* Miers (1), *Pachygone* Miers (1), *Parabaena* Miers (1), *Pericampylus* Miers (1), *Pycnarrhena* Miers ex Hook. f. & Thomson (1), *Stephania* Lour. (9), *Tinomiscium* Miers ex Hook. f. & Thomson (1), *Tinospora* Miers (3)
Menyanthaceae (1)	*Nymphoides* Ség. (1)
Moraceae (7)	*Antiaris* Lesch. (1), *Artocarpus* J. R. Forst. & G. Forst. (5), *Broussonetia* L'Hér. ex Vent. (2), *Ficus* L. (58), *Maclura* Nutt. (3), *Morus* L. (2), *Streblus* Lour. (2)
Musaceae (2)	*Ensete* Horan. (1), *Musa* L. (5)
Myricaceae (1)	*Myrica* L. (1)
Myristicaceae (3)	*Horsfieldia* Willd. (4), *Knema* Lour. (4), *Myristica* Gronov. (1)
Myrsinaceae (4)	*Ardisia* Sw. (10), *Embelia* Burm. f. (8), *Maesa* Forssk. (8), *Myrsine* L. (2)
Myrtaceae (2)	*Decaspermum* J. R. Forst. & G. Forst. (1), *Syzygium* P. Browne ex Gaertn. (22)
Najadaceae (1)	*Najas* L. (1)
Nyctaginaceae (1)	*Pisonia* L. (1)
Nymphaeaceae (2)	*Brasenia* Schreb. (1), *Nuphar* Sm. (1)
Nyssaceae (2)	*Camptotheca* Decne. (1), *Nyssa* L. (3)
Olacaceae (3)	*Erythropalum* Blume (1), *Olax* L. (1), *Schoepfia* Schreb. (1)
Oleaceae (7)	*Chionanthus* L. (1), *Fraxinus* L. (2), *Jasminum* L. (10), *Ligustrum* L. (4), *Linociera* Sw. ex Schreb. (1), *Olea* L. (3), *Osmanthus* Lour. (2)
Onagraceae (2)	*Epilobium* L. (1), *Ludwigia* L. (5)
Opiliaceae (2)	*Opilia* Roxb. (1), *Urobotrya* Stapf (1)
Orchidaceae (94)	*Acampe* Lindl. (2), *Acanthephippium* Blume ex Endl. (2), *Acriopsis* Blume (1), *Aerides* Lour. (2), *Agrostophyllum* Blume (1), *Ania* Lindl. (3), *Anoectochilus* Blume (3), *Anthogonium* Wall. ex Lindl. (1), *Aphyllorchis* Blume (2), *Apostasia* Blume (1), *Arachnis* Blume (1), *Arundina* Blume (1), *Ascocentrum* Schltr. ex J. J. Sm. (1), *Brachycorythis* Lindl. (1), *Bulbophyllum* Thouars (40), *Calanthe* R. Br. (5), *Callostylis* Blume (1), *Cephalantheropsis* Guillaumin (1), *Ceratostylis* Blume (1),

Appendix 1. Continued.

Families	Genera
	Cheirostylis Blume (2), *Chiloschista* Lindl. (1), *Chrysoglossum* Blume (1), *Cleisostoma* Blume (10), *Coelogyne* Lindl. (9), *Corymborchis* Thouars (1), *Cymbidium* Sw. (10), *Dendrobium* Sw. (43), *Diploprora* Hook. f. (1), *Epipactis* Zinn (1), *Epipogium* J. F. Gmel. ex Borkh. (2), *Eria* Lindl. (22), *Eriodes* Rolfe (1), *Erythrodes* Blume (1), *Esmeralda* Rchb. f. (1), *Eulophia* R. Br. ex Lindl. (1), *Flickingeria* A. D. Hawkes (6), *Galeola* Lour. (2), *Gastrochilus* D. Don (5), *Gastrodia* R. Br. (1), *Geodorum* Jacks. (2), *Goodyera* R. Br. (3), *Habenaria* Willd. (12), *Hemipilia* Lindl. (1), *Herminium* L. (1), *Hetaeria* Blume (1), *Holcoglossum* Schltr. (2), *Hygrochilus* Pfitzer (1), *Kingidium* P. F. Hunt (1), *Lecanorchis* Blume (1), *Liparis* Rich. (11), *Luisia* Gaudich. (4), *Malaxis* Sol. ex Sw. (9), *Nervilia* Commons ex Gaudich. (2), *Oberonia* Lindl. (13), *Ornithochilus* (Lindl.) Wall. ex Benth. (1), *Otochilus* Lindl. (2), *Panisea* Lindl. (1), *Paphiopedilum* Pfitzer (2), *Papilionanthe* Schltr. (2), *Parapteroceras* Aver. (1), *Pecteilis* Raf. (2), *Pelatantheria* Ridl. (3), *Pennilabium* J. J. Sm. (1), *Peristylus* Blume (4), *Phaius* Lour. (6), *Phalaenopsis* Blume (1), *Pholidota* Lindl. ex Hook. (4), *Phreatia* Lindl. (1), *Platanthera* Rich. (1), *Pleione* D. Don (1), *Podochilus* Blume (1), *Polystachya* Hook. (1), *Porpax* Lindl. (1), *Pteroceras* Hasselt ex Hassk. (1), *Rhynchostylis* Blume (1), *Robiquetia* Gaudich. (1), *Sarcoglyphis* Garay (1), *Schoenorchis* Blume (2), *Spathoglottis* Blume (1), *Spiranthes* Rich. (1), *Staurochilus* Ridl. ex Pfitzer (2), *Sunipia* Lindl. (5), *Taeniophyllum* Blume (2), *Tainia* Blume (2), *Thelasis* Blume (1), *Thrixspermum* Lour. (2), *Thunia* Rchb. f. (1), *Trichoglottis* Blume (1), *Tropidia* Lindl. (2), *Uncifera* Lindl. (1), *Vanda* R. Br. (1), *Vandopsis* Pfitzer (2), *Vanilla* Plum. ex Mill. (1), *Zeuxine* Lindl. (3)
Orobanchaceae (1)	*Aeginetia* L. (1)
Oxalidaceae (3)	*Averrhoa* L. (1), *Biophytum* DC. (3), *Oxalis* L. (1)
Pandanaceae (1)	*Pandanus* Parkinson (1)
Papaveraceae (1)	*Argemone* L. (1)
Passifloraceae (2)	*Adenia* Forssk. (2), *Passiflora* L. (4)
Pinaceae (1)	*Pinus* L. (1)
Piperaceae (3)	*Peperomia* Ruiz & Pav. (5), *Piper* L. (27), *Zippelia* Blume (1)
Pittosporaceae (1)	*Pittosporum* Banks ex Gaertn. (6)
Plantaginaceae (1)	*Plantago* L. (3)
Poaceae (63)	*Acroceras* Stapf (1), *Apluda* L. (1), *Arthraxon* P. Beauv. (1), *Arundinella* Raddi (2), *Arundo* L. (1), *Bambusa* Schreb. (3), *Bothriochloa* Kuntze (1), *Brachiaria* (Trin.) Griseb. (1), *Centotheca* Desv. (1), *Cephalostachyum* Munro (2), *Chimonobambusa* Makino (2), *Chloris* Sw. (1), *Chrysopogon* Trin. (1), *Coix* L. (1), *Cymbopogon* Spreng. (2), *Cynodon* Rich. (2), *Cyrtococcum* Stapf (1), *Dendrocalamus* Nees (20), *Digitaria* Haller (4), *Echinochloa* P. Beauv. (2), *Eleusine* Gaertn. (2), *Eragrostis* Wolf (6), *Erianthus* Michx. (1), *Eriochloa* Kunth (1), *Fargesia* Franch. (1), *Gigantochloa* Kurz ex Munro (6), *Hackelochloa* Kuntze (1), *Hymenachne* P. Beauv. (1), *Hyparrhenia* Andersson ex E. Fourn. (1), *Imperata* Cirillo (1), *Indosasa* McClure (3), *Isachne* R. Br. (1), *Ischaemum* L. (1), *Leptochloa* P. Beauv. (1), *Lophatherum* Brongn. (1), *Melocalamus* Benth. (3), *Microstegium* Nees (1), *Miscanthus* Andersson (1), *Neyraudia* Hook. f. (1), *Oplismenus* P. Beauv. (2), *Oryza* L. (2), *Panicum* L. (3), *Paspalidium* Stapf (1), *Paspalum* L. (5), *Phyllostachys* Siebold & Zucc. (3), *Pleioblastus* Nakai (1), *Poa* L. (1), *Pogonatherum* P. Beauv. (1), *Pseudechinolaena* Stapf (1), *Pseudostachyum* Munro (1), *Rottboellia* L. f. (1), *Saccharum* L. (2), *Sacciolepis* Nash (3), *Schizostachyum* Nees (3), *Setaria* P. Beauv. (4), *Sorghum* Moench (2), *Sporobolus* R. Br. (1), *Stenotaphrum* Trin. (1), *Themeda* Forssk. (5), *Thyrsostachys* Gamble (2), *Thysanolaena* Nees (1), *Urochloa* P. Beauv. (1), *Yushania* Keng f. (1)
Podocarpaceae (3)	*Dacrycarpus* (Endl.) de Laub. (1), *Nageia* Gaertn. (2), *Podocarpus* L'Hér. ex Pers. (1)
Podostemaceae (2)	*Cladopus* H. Möller (1), *Hydrobryum* Endl. (1)
Polygalaceae (4)	*Polygala* L. (13), *Salomonia* Lour. (4), *Securidaca* L. (1), *Xanthophyllum* Roxb. (2)
Polygonaceae (4)	*Fagopyrum* Mill. (1), *Polygonum* L. (16), *Reynoutria* Houtt. (1), *Rumex* L. (1)
Pontederiaceae (1)	*Monochoria* C. Presl (3)
Portulacaceae (2)	*Portulaca* L. (1), *Talinum* Adans. (1)
Potamogetonaceae (1)	*Potamogeton* L. (2)
Primulaceae (1)	*Lysimachia* L. (9)
Proteaceae (2)	*Helicia* Lour. (6), *Heliciopsis* Sleumer (2)
Rafflesiaceae (1)	*Sapria* Griff. (1)
Ranunculaceae (4)	*Anemone* L. (2), *Clematis* L. (9), *Naravelia* Adans. (1), *Ranunculus* L. (2)
Rhamnaceae (9)	*Berchemia* Neck ex DC. (3), *Chaydaia* Pit. (1), *Gouania* Jacq. (4), *Hovenia* Thunb. (1), *Rhamnus* L. (2), *Sageretia* Brongn. (2), *Scutia* (Comm. ex A. DC.) Brongn. (1), *Ventilago* Gaertn. (5), *Ziziphus* Mill. (6)

Appendix 1. Continued.

Families	Genera
Rhizophoraceae (2)	*Carallia* Roxb. (1), *Pellacalyx* Korth. (1)
Rosaceae (18)	*Agrimonia* L. (2), *Cerasus* Mill. (1), *Chaenomeles* Lindl. (1), *Dichotomanthes* Kurz (1), *Docynia* Decne. (1), *Duchesnea* Sm. (2), *Eriobotrya* Lindl. (2), *Laurocerasus* Duhamel (4), *Neillia* D. Don (1), *Photinia* Lindl. (5), *Potentilla* L. (3), *Pygeum* Gaertn. (3), *Pyracantha* M. Roem. (1), *Pyrus* L. (2), *Rosa* L. (1), *Rubus* L. (10), *Sorbus* L. (5), *Stranvaesia* Lindl. (1)
Rubiaceae (45)	*Aidia* Lour. (2), *Borreria* G. Mey. (1), *Brachytome* Hook. f. (2), *Canthium* Lam. (3), *Cephalanthus* L. (1), *Chassalia* DC. (1), *Dentella* J. R. Forst. & G. Forst. (1), *Diplospora* DC. (2), *Duperrea* Pierre ex Pit. (1), *Gardenia* J. Ellis (1), *Geophila* D. Don (1), *Hedyotis* L. (14), *Hymenodictyon* Wall. (3), *Hyptianthera* Wight & Arn. (1), *Ixora* L. (6), *Knoxia* L. (1), *Lasianthus* Jack (12), *Litosanthes* Blume (1), *Metadina* Bakh. f. (1), *Mitragyna* Korth. (1), *Morinda* L. (3), *Mussaenda* L. (7), *Mycetia* Reinw. (9), *Myrioneuron* R. Br. ex Kurz (1), *Neanotis* W. H. Lewis (4), *Neolamarckia* Bosser (1), *Neonauclea* Merr. (3), *Ophiorrhiza* L. (13), *Oxyceros* Lour. (3), *Paederia* L. (2), *Pavetta* L. (4), *Prismatomeris* Thwaites (1), *Psychotria* L. (6), *Randia* L. (1), *Rubia* L. (2), *Saprosma* Blume (1), *Schizomussaenda* H. L. Li (1), *Sinoadina* Ridsdale (1), *Spiradiclis* Blume (2), *Tarenna* Gaertn. (1), *Tarennoidea* Tirveng. & Sastre (2), *Uncaria* Schreb. (5), *Urophyllum* Jack ex Wall. (1), *Wendlandia* Bartl. ex DC. (11), *Xeromphis* Raf. (1)
Rutaceae (14)	*Acronychia* J. R. Forst. & G. Forst. (1), *Atalantia* Corrêa (1), *Boenninghausenia* Rchb. ex Meisn. (1), *Citrus* L. (1), *Clausena* Burm. f. (5), *Euodia* J. R. Forst. & G. Forst. (8), *Fortunella* Swingle (1), *Glycosmis* Corrêa (6), *Micromelum* Blume (3), *Murraya* J. König ex L. (2), *Paramignya* Wight (1), *Skimmia* Thunb. (1), *Toddalia* Juss. (1), *Zanthoxylum* L. (8)
Sabiaceae (2)	*Meliosma* Blume (5), *Sabia* Colebr. (3)
Salicaceae (1)	*Salix* L. (1)
Santalaceae (5)	*Dendrotrophe* Miq. (1), *Osyris* L. (1), *Phacellaria* Benth. (2), *Pyrularia* Michx. (1), *Scleropyrum* Arn. (1)
Sapindaceae (11)	*Allophylus* L. (3), *Arytera* Blume (1), *Cardiospermum* L. (1), *Dodonaea* Mill. (1), *Harpullia* Roxb. (1), *Litchi* Sonn. (1), *Mischocarpus* Blume (1), *Nephelium* L. (1), *Pometia* J. R. Forst. & G. Forst. (1), *Sapindus* L. (1), *Xerospermum* Blume (1)
Sapotaceae (3)	*Pouteria* Aubl. (1), *Sarcosperma* Hook. f. (4), *Xantolis* Raf. (3)
Saururaceae (2)	*Houttuynia* Thunb. (1), *Saururus* L. (1)
Saxifragaceae (3)	*Bergenia* Moench (1), *Itea* L. (2), *Tiarella* L. (1)
Scrophulariaceae (15)	*Adenosma* R. Br. (3), *Alectra* Thunb. (1), *Bacopa* Aubl. (1), *Brandisia* Hook. f. & Thomson (2), *Centranthera* R. Br. (2), *Dopatrium* Buch.-Ham. ex Benth. (1), *Limnophila* R. Br. (4), *Lindenbergia* Lehm. (2), *Lindernia* All. (10), *Mazus* Lour. (1), *Microcarpaea* R. Br. (1), *Picria* Lour. (1), *Torenia* L. (2), *Veronica* L. (1), *Wightia* Wall. (1)
Simaroubaceae (3)	*Ailanthus* Desf. (2), *Brucea* J. F. Mill. (2), *Picrasma* Blume (1)
Smilacaceae (1)	*Smilax* L. (15)
Solanaceae (4)	*Lycianthes* (Dunal) Hassl. (6), *Nicandra* Adans. (1), *Physalis* L. (2), *Solanum* L. (15)
Sonneratiaceae (1)	*Duabanga* Buch.-Ham. (1)
Sparganiaceae (1)	*Sparganium* L. (1)
Sphenocleaceae (1)	*Sphenoclea* Gaertn. (1)
Stachyuraceae (1)	*Stachyurus* Siebold & Zucc. (1)
Staphyleaceae (2)	*Tapiscia* Oliv. (1), *Turpinia* Vent. (4)
Stemonaceae (1)	*Stemona* Lour. (2)
Sterculiaceae (12)	*Ambroma* L. f. (1), *Byttneria* Loefl. (3), *Eriolaena* DC. (4), *Erythropsis* Lindl. ex Schott & Endl. (2), *Helicteres* L. (6), *Heritiera* Aiton (1), *Melochia* L. (1), *Pterospermum* Schreb. (5), *Pterygota* Schott & Endl. (1), *Reevesia* Lindl. (3), *Sterculia* L. (5), *Waltheria* L. (1)
Stylidiaceae (1)	*Stylidium* Sw. ex Willd. (1)
Styracaceae (4)	*Alniphyllum* Matsum. (1), *Bruinsmia* Boerl. & Koord. (1), *Huodendron* Rehder (1), *Styrax* L. (4)
Symplocaceae (1)	*Symplocos* Jacq. (9)
Taccaceae (1)	*Tacca* J. R. Forst. & G. Forst. (1)
Theaceae (9)	*Adinandra* Jack (1), *Anneslea* Wall. (1), *Camellia* L. (6), *Eurya* Thunb. (5), *Gordonia* J. Ellis (1), *Pyrenaria* Blume (3), *Schima* Reinw. ex Blume (2), *Sladenia* Kurz (1), *Ternstroemia* Mutis ex L. f. (2)
Thymelaeaceae (2)	*Aquilaria* Lam. (1), *Eriosolena* Blume (1)
Tiliaceae (5)	*Colona* Cav. (2), *Corchorus* L. (3), *Grewia* L. (6), *Microcos* L. (2), *Triumfetta* L. (4)
Trapaceae (1)	*Trapa* L. (1)
Ulmaceae (5)	*Aphananthe* Planch. (2), *Celtis* L. (3), *Gironniera* Gaudich. (1), *Trema* Lour. (3), *Ulmus* L. (1)

Appendix 1. Continued.

Families	Genera
Urticaceae (14)	*Boehmeria* Jacq. (13), *Debregeasia* Gaudich. (4), *Dendrocnide* Miq. (3), *Elatostema* J. R. Forst. & G. Forst. (14), *Girardinia* Gaudich. (1), *Gonostegia* Turcz. (1), *Maoutia* Wedd. (1), *Oreocnide* Miq. (3), *Pellionia* Gaudich. (6), *Pilea* Lindl. (17), *Poikilospermum* Zipp. ex Miq. (2), *Pouzolzia* Gaudich. (4), *Procris* Comm. ex Juss. (1), *Urtica* L. (2)
Valerianaceae (1)	*Triplostegia* Wall. ex DC. (1)
Verbenaceae (11)	*Callicarpa* L. (11), *Caryopteris* Bunge (1), *Clerodendrum* L. (14), *Congea* Roxb. (2), *Garrettia* H. R. Fletcher (1), *Gmelina* L. (1), *Phyla* Lour. (1), *Premna* L. (8), *Sphenodesme* Jack (1), *Symphorema* Roxb. (1), *Vitex* L. (7)
Violaceae (1)	*Viola* L. (9)
Viscaceae (2)	*Korthalsella* Tiegh. (1), *Viscum* L. (6)
Vitaceae (7)	*Ampelopsis* Michx. (4), *Cayratia* Juss. (4), *Cissus* L. (9), *Iæea* D. Royen ex L. (7), *Tetrastigma* (Miq.) Planch. (16), *Vitis* L. (4), *Yua* C. L. Li (1)
Xyridaceae (1)	*Xyris* L. (2)
Zingiberaceae (17)	*Alpinia* Roxb. (11), *Amomum* Roxb. (14), *Boesenbergia* Kuntze (1), *Cautleya* Hook. f. (1), *Costus* L. (3), *Curcuma* L. (9), *Curcumorpha* A. S. Rao & D. M. Verma (1), *Etlingera* Giseke (1), *Globba* L. (4), *Hedychium* J. König (7), *Kaempferia* L. (3), *Paramomum* S. Q. Tong (1), *Pommereschea* Wittm. (2), *Rhynchanthus* Hook. f. (1), *Siliquamomum* Baill. (1), *Stahlianthus* Kuntze (1), *Zingiber* Mill. (11)
Zygophyllaceae (1)	*Tribulus* L. (1)

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- ☐ Electronic scans of figures are included with the submission, with the understanding that these may have to be revised based on review. Low-resolution JPEG images are suitable for the review process, but are not acceptable for final submission to the printer. See the printer's electronic illustration requirements. Go to http://www.mbgpress.org/; click on the Publication Submissions at upper right of opening page; click on Guide to Digital Art, Allen Press.
- ☐ Authorities, abbreviated according to Brummit & Powell's *Authors of Plant Names*, are provided for species' names the first time they are mentioned in the text; OR they are provided in a table that includes names of species.
- ☐ Email or letter is enclosed containing the author's full mailing address, telephone number, fax number, and e-mail address (required).

2. Format of Text

- ☐ Right-hand margin is not justified, and words are not hyphenated there.
- ☐ One space is inserted after periods, colons, question marks, and all other punctuation marks.
- ☐ First sentences of paragraphs are indented with a tab.
- ☐ Two hyphens with no space between them are used to indicate ranges of numbers, for example, page numbers, 20–23; measurements, 5–7 mm; altitudes, 1500–2500 m.
- ☐ A single hyphen is used in hyphenated words, for example, 9-nerved, oval-triangular.
- ☐ Common Latin words or phrases are not italicized (e.g., et al., i.e., sensu, etc.).
- ☐ Only taxon names at the rank of genus and below are italicized.
- ☐ Correct accents, umlauts, and other diacritical marks have been included.
- ☐ All figures and tables are cited in the text and are numbered in the order in which they are to appear.

3. Style

- ☐ Recent issue of the *Annals* is used as a model.
- ☐ *Chicago Manual of Style*, latest edition, is used as a reference.

4. First Page

- ☐ Footnotes are typed as double-spaced paragraphs on the first page. The first footnote contains acknowledgments, including information on granting agencies, herbaria that loaned specimens, and the name of the artist. The second footnote is the author's address.

Addresses for additional authors are each separate footnotes. No other footnotes occur, except in tables, where unavoidable.

5. Abstract & Key Words

[ ] A one-paragraph abstract precedes the text. Papers in Spanish have an English abstract in addition to a Spanish resumen.

[ ] The abstract is concise (1 paragraph) and includes brief statements about the paper's intent, materials and methods, results, and significance of findings.

☐ A brief list of key words immediately follows the Abstract.

6. Taxonomic Treatment

[ ] Species entries are organized as follows: Heading, vernacular name(s), Latin diagnosis (if necessary), description, distribution, summary, discussion, specimens examined. The discussions are parallel and follow the same order, e.g., diagnostic characteristics, distinction from similar species variation, distribution and ecology, nomenclature and typification, uses.

☐ One paragraph per basionym is used as follows: Taxon author, literature citation, type citation, e.g., *Beilschmiedia latifolia* (Nees) Sa. Nishida, Ann. Missouri Bot. Gard. 86: 680. 1999. *Hufelandia latifolia* Nees, Syst. Laur.: 674. 1836. TYPE: Peru. Locality not indicated, 1835 (fl), *Matthew 1433* (holotype, E!; isotypes, BM!, E!, K!, LE not seen, OXF not seen).

[ ] Synonyms based on different types are placed in separate paragraphs, each beginning with the basionym, followed by other combinations (if appropriate), and citation of the type.

[ ] A brief Latin diagnosis for each new taxon is provided rather than a complete Latin description.

[ ] For species with infraspecific taxa: Description and discussion are composite (incorporating all infraspecific taxa) and parallel with other species descriptions. Descriptions of infraspecific taxa are parallel with one another (in the same species). All synonyms are listed under the appropriate infraspecific taxon.

[ ] Descriptions: Descriptions are parallel, within a given rank. All measurements are metric. Hyphens are used for parenthetical extremes: "peduncle (8.2 -)14.3–28.0(–31.9) cm long," unless intermediate values are not expected: ovary with (2)4(6) locules. Length × width are given in the following manner: lamina 36.4–82.8 × 9.1–16.8 cm.

[ ] When relevant, nomina nuda, misapplied names, and superfluous names are included in the discussion following the description, but are not part of the formal synonymy.

7. Citation of Types

[ ] Exclamation points are used for specimens examined, and types not seen are indicated as such (e.g., MO!, US not seen).

[ ] Lectotype designations are included together with an indication of where they were designated [author, year, page number, and herbarium of deposition; e.g., *C. J. W. Schiede 159* (lectotype, designated by Stevens (2000: 256), P!; isotypes. . . .)]. This reference is listed in the Literature Cited. If the author of the paper submitted is making the lectotypification, the phrase "designated here" is used.

8. Tables

Create tables using the Microsoft Word "Table" feature.

[ ] Tables are neat, double-spaced, and clearly presented.

[ ] Captions are typed double-spaced as paragraphs at the tops of the tables.

[ ] Each table starts on a separate sheet.

9. Abbreviations

[ ] Periods are used after all abbreviations (which are minimized) except metric measures, compass directions, and herbarium designations.

[ ] When dates are given as part of collection information, three-letter month abbreviations are used, except for months with four letters, which are spelled out in full.

[ ] States are not abbreviated, and cities are spelled out. [St., as in St. Louis, is acceptable.]

[ ] Periodicals are abbreviated according to *B-P-H* (*Botanico-Periodicum-Huntianum*) and to *B-P-H/S* (*Botanico-Periodicum-Huntianum/Supplementum*).

[ ] Authors' names are abbreviated according to Brummit & Powell's *Authors of Plant Names*.

[ ] Book titles are abbreviated according to *Taxonomic Literature, edition 2*, but with initial letters capitalized. Book titles are spelled out in the Literature Cited.

[ ] If an item does not appear in *B-P-H* or *TL-2*, or if these references are not available, its title is fully spelled out.

[ ] Herbaria are abbreviated according to the most recent edition of *Index Herbariorum*.

[ ] Abbreviated forms are not used for references in the text, except when citing the names of plants. If it is necessary to cite a particular page in the text, the form Smith (1998: 12) is used.

10. Specimens Examined

[ ] If many specimens were examined, those cited in the text are limited to ca. 12 manuscript pages.

[ ] If there are a large number, an index to specimens examined is placed at the end of the paper, following the Literature Cited. It is arranged alphabetically by collector, followed by collection number, followed by the number of the taxon in the text. Names (including initial(s)) of first and second collector are provided, "et al." if three or more.

[ ] Specimens are cited in the text as follows: *Additional specimens examined* (or *Selected specimens examined*). MEXICO. **Oaxaca:** Sierra San Pedro Nolesco, Talea, 12°37′N, 85°14′W, 950–1100 m, 3 Feb. 1987 (fl), *Jergensen 865* (BM, G, K, US). [Dates and reproductive status are optional but are omitted from longer lists.] Countries are run together in the same paragraph, e.g., COUNTRY A. **Major political division:** . . . COUN-

TRY B. **Major political division:** ...Separate paragraphs are used for major continental regions within major political divisions.

11. Specimen Vouchers and Genetic Sequences

[ ] If the paper presents original data, associated herbarium vouchers are cited. [Vouchers for seed and/or other collections should be included where pertinent. Dependent on the paper, reference to the original wild source may be required.] Vouchers are also cited from common names, and uses are taken from specimen labels.

[ ] Herbarium vouchers state the collector and number, herbarium in which the voucher is located, and a clear annotation that the material represents the voucher for the study in question.

☐ Nucleic acid or protein sequences corresponding to equal or greater than 50 nucleotides are entered into an appropriate data bank, e.g., GenBank/EMBL. The accession numbers are provided before publication. [Long sequences (exceeding two pages) will not be routinely published.]

☐ Author accepts responsibility for establishing the accuracy of information provided.

12. Keys

☐ Keys are clear and have been checked carefully for consistency with the descriptions. Leads of each couplet are parallel.

☐ Dichotomous keys are indented.

☐ Infraspecific taxa are keyed separately, not in species keys.

13. Literature Cited

☐ The Literature Cited contains full citations of all references cited in the text.

☐ All entries in the Literature Cited are cited in the text.

☐ Spelling of author(s) name(s) and years of publication have been double-checked.

☐ All entries have been verified against original sources, especially journal titles, accents, diacritical marks, and spelling in languages other than English.

☐ Periodicals are listed as follows: author's last name, initial(s). Year. Full title of article. Journal abbreviated as in *B-P-H/S*. Volume: pages. No parenthetical part numbers after volume numbers are given unless each part is paginated separately.

☐ For more than one author, this style is followed: author's last name, initial(s), second author's initial(s), last name & third author's initial(s), last name.

☐ Books appear as follows: author's last name, initial(s). Year. Full Unabbreviated Title (edited by Editor), 3rd ed., Vol. 2. Publisher, City of Publication.

☐ For an article within a larger work, this style is followed: Author(s). Year. Name of the article. Pp. 00–00 in Name of the editor(s), Full Title of Larger Work. Publisher, City of Publication.

☐ Citations of work "in prep.," unpublished theses and dissertations, and similar references to inaccessible sources have been eliminated or kept to a minimum. They are not necessarily included in the Literature Cited.

14. Illustrations

☐ Electronic figures are labeled with the first author's last name, the first four letters of the taxon, and "...Figure1.tif", "...Figure2.tif", etc. The file extension clearly indicates what type of file it is.

☐ Scale bars appear on illustrations, photographs, and maps.

☐ Magnifications/reductions are not indicated in captions.

☐ Electronic scans may be pre-sized to fit either column width (2-5/8 in. or ca. 68 mm) or full-page width (5-1/2 in. or ca. 140 mm). [Maximum size for printed illustrations is 5-1/2 × 8-1/4 in.]

☐ Figures are numbered in Arabic numerals in the order of their citation in the text. Parts of figures are labeled with capital letters.

☐ Photographs are prepared according to the printer's requirements. [See Guide to Digital Art, Allen Press through the Publication Submissions link on the opening page of http://www.mbgpress.org/.]

☐ Figures are grouped into composite plates when possible; edges of photographs are abutted.

☐ No stripping is inserted between plate or figure segments. [Printer will insert stripping.]

☐ Edges of figures are squared.

☐ Maps include reference to latitude and longitude and are bounded by a fine border.

☐ Scanning electron micrographs are free of conspicuous charging.

☐ Axes on graphs are all labeled.

☐ Captions provide all explanatory text. Captions are separate from other text, one paragraph for each group of figures, and following the style in current issues of the *Annals*.

☐ Symbols on maps are legible, and reduction has been taken into consideration.

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